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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a **PROVISIONAL APPLICATION FOR PATENT** under 37 CFR 1.53 (b)(2).

Docket Number	27066	Type a plus sign (+) inside this box ->	+

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TITLE OF THE INVENTION (280 characters max)

ANGIOGENIC PEPTIDES AND USES THEREOF

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification	*Number of Pages*	64	☑ Applicant is entitled to Small Entity Status	
☑ Drawing(s)	*Number of Sheets*	38	☑ Other (specify) ASSIGNMENT	
			5 PAGES SEQUENCE LISTING	36 CLAIMS

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT *(check one)*

☐ A check or money order is enclosed to cover the filing fees		FILING FEE AMOUNT ($)	$ 80.-
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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☑ No

☐ Yes, the name of the US Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE _____ Date March 31, 2004

25,457
REGISTRATION NO. *(if appropriate)*

TYPED or PRINTED NAME SOL SHEINBEIN

☐ Additional inventors are being named on separately numbered sheets attached hereto

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

APPLICATION FOR PATENT

Inventors: Britta Hardy, Alexander Battler, Annat Raiter and Chana Weiss

Title: ANGIOGENIC PEPTIDES AND USES THEREOF

FIELD AND BACKGROUND OF THE INVENTION

The present invention relates to peptides, which are capable of promoting angiogenesis and to the use thereof in the treatment of angiogenesis-dependent diseases, such as ischemic vascular diseases.

Angiogenesis is the process of generating new capillary blood vessels involving an interplay between cells and soluble factors (1). In brief, activated endothelial cells migrate and proliferate to form new vessels, which are surrounded by layers of periendothelial cells, including pericytes for small blood vessels and smooth muscle cells for large blood vessels.

The factors which regulate the angiogenic process are numerous and varied including soluble factors and tissue oxygen. In the past two decades, a number of angiogenic molecules which positively regulate the angiogenic process were successfully elucidated. These include Vascular Endothelium Growth Factor (VEGF), basic Fibroblast Growth Factor (bFGF), acidic FGF/FGF-1, hypoxia inducible factor 1-α (HIF 1α), and others (2). As mentioned, oxygen conditions have also important implications to the physiological and pathological angiogenic process (3). Under hypoxic conditions, VEGF gene expression is induced in endothelial cells and in pericytes to produce secretory forms of VEGF. VEGF in turn, may bind to VEGF receptor-2 (Kdr) or VEGF receptor-1 (Flt-1) expressed on endothelial cells in an autocrine or paracrine manner, thereby causing proliferation of endothelial cells, which may lead to angiogenesis. Basal amounts of vascular VEGF synthesized in normoxic states promote the maintenance of microvascular homeostasis (5). Expression of mRNA VEGF receptor 1 (Flt-1) was found to be up-regulated in peri-ischemic endothelial cells and in the infracted core of endothelial cells and periphery, with peak expression of VEGFR-1 in endothelial cells. Gene expression of VEGF-1 is directly inducible by hypoxia, as in the case of VEGF. Twenty-four hours following

hypoxia-induced VEGF gene expression, concurrently with the expression of the VEGFR-1 and 2 (Kdr) genes, endothelial cells start to proliferate (6, 7).

Hypoxia inducible gene products that participate in these responses include erytropoietin, vascular endothelial growth factor (VEGF) and glycolitic enzymes (8). Hypoxia can directly enhance the expression of bFGF mRNA in pericytes. Increased expression of bFGF may play an important role in the process of pericyte proliferation and in the differentiation of pericytes and smooth muscle cells (9).

Angiogenesis-dependent diseases result when the angiogenic process is disregulated resulting in excessive new blood vessels or insufficient blood vessels. Insufficient angiogenesis is related to a large number of diseases and conditions such as coronary artery diseases and delayed wound healing. To date, cardiovascular diseases are the leading cause of mortality in the United States, Europe, and Israel. In the United States, approximately one million deaths per year are attributed to the cardiac causes of which fifty percent are attributed to Coronary Artery Disease (CAD). The major morbidity from CAD is a result of obstructive coronary artery narrowing and the resultant myocardial ischemia. CAD affects more than 13 million people, and its annual economic burden is in excess of sixty billion U.S. Dollars.

Mechanical revascularization of obstructive coronary stenoses by percutaneous techniques including percutaneous transluminal angioplasty and stent implantation is used to restore normal coronary artery blood flow. In addition, coronary artery occlusion bypass surgery is performed using arterial and venous conduits as grafts into the coronary arterial tree. These treatment modalities have significant limitations in individuals with diffuse atherosclerotic disease, severe small vessel coronary artery disease, diabetic patients, or individuals having already undergone surgical or percutaneous procedures.

For these reasons therapeutic angiogenesis, aimed at stimulating new blood vessel growth is highly desirable. The therapeutic concept of angiogenesis therapy is based on the premise that the existing potential for vascular growth inherent to vascular tissue can be utilized to promote the development of new blood vessels under the influence of the appropriate angiogenic molecules.

Therapeutic angiogenesis defines the intervention used to treat local hypovascularity by stimulating or inducing neovascularization for the treatment of ischemic vascular disease.

Animal studies have proven the feasibility of enhancing collateral perfusion and function via angiogenic compounds. Those experiments proved that exogenous administration of angiogenic growth factors or their genetic constructs could promote collateral vessel growth in experimental models of chronic ischemia. Although such studies demonstrated proof of concept, additional studies raise issues that still have not been resolved such as the duration of exposure of the vessels to angiogenic factors and the brief half-lives of proteins (10).

The study of synthetic peptides encompassing portions of proteins has turned into a supportive tool for understanding the molecular mechanism associated with protein biological functions. The use of short peptides, constructed from specific regions of human FGF and VEGF that have the potential to efficiently agonize or antagonize the biological functions of the growth factors family members have been described (11). Several groups have reported the use of intact cells to screen a phage display peptide library to identify cell surface binding peptides (12). It was reported that a peptide library was screened on human endothelial cells stimulated with VEGF to construct a peptide-based ligand receptor map of the VEGF family (13). Another study has described the screening of a 12-mer phage display peptide library on VEGF-2 receptor protein (14).

While reducing the present invention to practice, the present inventors used a 12-mer phage display peptide library to uncover peptides which are able to bind the cell-surface of endothelial cells incubated under normoxic or hypoxic conditions. Such peptides were shown to trigger angiogenic processes including, endothelial cell-proliferation and vascularization. As such, these peptides can be used to treat various angiogenesis-dependent diseases, such as ischemic vascular diseases. Furthermore, characterizing the nature of endothelial cell signaling by these peptides will provide the basis for the development of targeted angiogenic therapy for diseases such as cardiovascular disease.

SUMMARY OF THE INVENTION

According to one aspect of the present invention there is provided a peptide comprising an amino acid sequence as set forth in SEQ ID NO: 13, the peptide being at least 6 and no more than 50 amino acid residues in length.

According to another aspect of the present invention there is provided a peptide selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10 and 12.

According to yet another aspect of the present invention there is provided a peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10 and 12, the peptide being no more than 50 amino acid residues in length.

According to still another aspect of the present invention there is provided a composition-of-matter comprising at least two peptides, each independently selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10 and 12.

According to an additional aspect of the present invention there is provided a pharmaceutical composition comprising a therapeutically effective amount of a peptide having an amino acid sequence as set forth in SEQ ID NO: 13, the peptide being at least 6 and no more than 50 amino acid residues in length and a pharmaceutically acceptable carrier or diluent.

According to yet an additional aspect of the present invention there is provided a pharmaceutical composition comprising a therapeutically effective amount of a peptide selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10 and 12 and a pharmaceutically acceptable carrier or diluent.

According to still an additional aspect of the present invention there is provided a pharmaceutical composition comprising a therapeutically effective amount of a peptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10 and 12, the peptide being no more than 50 amino acid residues in length and a pharmaceutically acceptable carrier or diluent.

According to a further aspect of the present invention there is provided a method of promoting angiogenesis in a tissue of a subject, the method comprising providing to the subject, a therapeutically effective amount of a peptide having an amino acid sequence as set forth in SEQ ID NO: 13, the peptide being at least 6 and no more than 50 amino acid residues in length, to thereby promote angiogenesis in the subject.

According to further features in preferred embodiments of the invention described below, the peptide is selected from the group consisting of SEQ ID NOs: 2, 6 and 12.

According to still further features in the described preferred embodiments the amino acid sequence is selected from the group consisting of SEQ ID NOs: 2, 6 and 12.

According to still further features in the described preferred embodiments the peptide is a linear peptide or a cyclic peptide.

According to yet a further aspect of the present invention there is provided a method of promoting angiogenesis in a tissue of a subject, the method comprising providing to the subject, a therapeutically effective amount of a peptide selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10 and 12, to thereby promote angiogenesis in the subject.

According to still a further aspect of the present invention there is provided a method of promoting angiogenesis in a tissue of a subject, the method comprising providing to the subject, a therapeutically effective amount of a peptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10 and 12, the peptide being no more than 50 amino acid residues in length, to thereby promote angiogenesis in the subject.

According to still further features in the described preferred embodiments the subject suffers from arteriosclerosis, retinopathy, remodeling disorder, von Hippel-Lindau syndrome, diabetes and/or hereditary hemorrhagic telengiectasia.

According to still a further aspect of the present invention there is provided a nucleic acid construct comprising a polynucleotide sequence encoding the peptide of the present invention.

According to still further features in the described preferred embodiments the nucleic acid construct further comprising a promoter.

According to still a further aspect of the present invention there is provided a composition for targeting a drug to endothelial cells, the composition comprising the drug fused to a peptide having an amino acid sequence as set forth in SEQ ID NO: 13, the peptide being at least 6 and no more than 50 amino acid residues in length.

According to still further features in the described preferred embodiments the drug is selected from the group consisting of a toxin, a chemotherapeutic agent and a radioisotope.

According to still a further aspect of the present invention there is provided a composition for targeting a drug to endothelial cells, the composition comprising the

drug fused to a peptide selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10 and 12.

According to still a further aspect of the present invention there is provided a composition for targeting a drug to endothelial cells, the composition comprising the drug fused to a peptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10 and 12, the peptide being no more than 50 amino acid residues in length.

According to still a further aspect of the present invention there is provided a method of identifying putative angiogenic molecules, the method comprising: (a) providing endothelial cells having peptides bound thereto, each of the peptides having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10 and 12, the peptide being no more than 50 amino acid residues in length; and (b) identifying a molecule capable of displacing the peptides from the endothelial cells, to thereby identify putative angiogenic molecules.

According to still a further aspect of the present invention there is provided an antibody or an antibody fragment comprising an antigen recognition region capable of binding the peptide of claim 1, 5, 7 or 9.

The present invention successfully addresses the shortcomings of the presently known configurations by providing peptides, which are capable of promoting angiogenesis, and as such can be used to treat angiogenesis-dependent diseases, such as ischemic vascular diseases.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of

illustrative discussion of the preferred embodiments of the present invention only, and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for a fundamental understanding of the invention, the description taken with the drawings making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

In the drawings:

FIGs. 1a-b are bar graphs depicting the binding of phage display peptides at a concentration of (Figure 1a) 10^9 phages or (Figure 1b) 10^{10} phages, to ECs at normal conditions and following 3, 6, and 24 hours of hypoxia. The bars compare 15 different phage display peptides (VL, LP, TR, ST, QF, NS, SP, YR, LT, HR, HY, SV, TP, NR and SA) binding to ECs as compared to control (NO, unmodified M13 phages) following 2 hours of incubation. Data was obtained by ELISA, in which Anti-M13 HRP antibody detects phage display peptides attached to ECs and in the presence of tetramethyl benzidine liquid substrate produces absorbance at 450nm. Data was collected using ELISA reader.

FIG. 2 is a bar graph depicting the effect of phage display peptides on ECs proliferation. Six phage display peptides (VL, TR, YR, QF, LT, SP) each at a concentration of 10^6 were incubated with ECs in serum free media for 24 hours. Data was obtained by measuring radioactive thymidine uptake into ECs (cpm/min) in the last 6 hours of incubation, and presented as a percent above ECs proliferation induced by control phages (NO, unmodified M13 phages).

FIGs. 3a-b are bar graphs depicting the effect of direct activation of phage display peptides on ECs migration. The migration of ECs was assayed in the presence of phage display peptides at a concentration of (Figure 3a) 10^5 phages or (Figure 3b) 10^6 phages as compared to negative controls (ECs, or ECs in the presence of NO phages - unmodified M13 phages) and positive control (the angiogenic molecule-VEGF). The bars compare ECs migration induced by 12 phage display peptides (VL, LP, QF, SP, TR, NS, SV, LA, LT, YR, TP and SA) following 5 hours of incubation in migration chambers. Data was obtained by measuring the fluorescent enhancement of the CyQuant GR dye molecular probe bound to cellular nucleic acid of lysed

migratory cells. Data was collected by a Fluorescent ELISA reader at 480/520nm and expressed as Relative Fluorescence Units (RFU).

FIGs. 4a-b are bar graphs depicting chemo-attraction of ECs by phage display peptides added to the feeder tray of the migration chamber. Phage display peptides were used at a concentration of (Figure 4a) 10^5 phages or (Figure 4b) 10^6 phages as compared to negative controls (ECs or ECs in the presence of NO phages - unmodified M13 phages). The bars compare ECs chemo-attraction induced by 12 phage display peptides (VL, LP, QF, SP, TR, NS, SV, LA, LT, YR, TP and SA) following 5 hours of incubation in migration chamber. Data was obtained by measuring the fluorescent enhancement of the CyQuant GR dye molecular probe bound to cellular nucleic acid of lysed migratory cells. Data was collected by a Fluorescent ELISA reader at 480/520nm and expressed as Relative Fluorescence Units (RFU).

FIG. 5 is a bar graph depicting the proliferation of ECs in arterial rings in the presence of phage display peptides (10^6) as compared with negative control (NO phage, unmodified M13 phages) and positive control (the angiogenic molecule-FGF). The bars compare ECs proliferation induced by six phage display peptides (SP, LT, YR, TR, VL, and QF) following 7 days incubation in DMEM containing 10 % FCS (37 °C with 5 % CO_2). Data was obtained by an XTT assay (O.D. 450 nm).

FIGs. 6a-i are graphs depicting the specific binding of synthetic peptides to (Figure 6a) Peripheral Blood Lymphocytes (PBL) (Figure 6b-i) or ECs. The graphs represent flow cytometry analysis of 100,000 cells incubated for 2 hours with 4 or 6 μg of synthetic peptides. Figures 6a-b are the results of flow cytometry analysis presenting the gates chosen either for (Figure 6a) peripheral blood lymphocytes or for (Figure 6b) endothelial cells analysis. The dots represent the dispersion of FITC labeled cells, according to their size (horizontal axis) versus their granulation (vertical axis). Figures 6c-i are the results of flow cytometry analysis presenting the percent binding and mean fluorescence of synthetic peptides (Figure 6c) no peptide, (Figure 6d) SP, (Figure 6e) QF, (Figure 6f) LT, (Figure 6g) YR, (Figure 6h) TR, and (Figure 6i) VL to ECs. The graphs represent flow cytometry analysis of 10^6 ECs incubated for 2 hours with 4 μg of synthetic peptides (green line), 6 μg of synthetic peptides (red line), or isotype control (black line).

FIG. 7 is a bar graph demonstrating binding of synthetic peptides to PBL and ECs. The graph represents flow cytometry analysis of 5 μg FITC labeled synthetic

peptide (SP, YR, LT, VL, QF and TR) bound to 100,000 PBLs and ECs. The bars compare mean fluorescence (emitted by the labeled synthetic peptides following 2 hours incubation with PBLs or ECs. Data was collected using FACS.

FIGs. 8a-b are graphs depicting the effect of synthetic peptides on cells proliferation. Figure 8a is illustrating the proliferation of ECs induced by LP, ST, TR, and VL at concentrations of 0.05, 0.1, 1, 10, and 100 ng/ml, following 24 hours incubation in EBM-2. Figure 8b is illustrating the proliferation of MVECs induced by LT SP, or YR at concentrations of 0.1, 1, 10, and 100 ng/ml following 24 hours incubation in EBM-MV. Results are expressed as thymidine uptake by cells incubated with peptides minus control (cells incubated in EBM-2 and EBM-MV, respectively). Data was obtained by measuring radioactive thymidine uptake into cells in a scintillation β counter by cpm/min in the last 6 hours of incubation.

FIGs. 9a-c are graphs depicting the effect of the synthetic peptides on ECs migration. Figure 9a-c are graphs illustrating the migration of ECs induced by (Figure 9a) LT (Figure 9b) SP, or (Figure 9c) VL and TR at concentrations of 5, 10, 20 and 50 ng/ml following 5 hours of incubation in migration chamber. Data was obtained by measuring the fluorescent enhancement of the CyQuant GR dye molecular probe bound to cellular nucleic acid of lysed migratory cells. Data was collected by a Fluorescent ELISA reader at 480/520nm and expressed as Relative Fluorescence Units (RFU).

FIG. 10 is a graph depicting the time dependent effect of peptide incubation on ECs migration. The figure presents the migration of ECs as induced by 1 ng/ml synthetic peptide (LT, QF, SP, TR, VL and YR) following 5 and 15 hours of incubation in migration chamber. Data was obtained by measuring the fluorescent enhancement of the CyQuant GR dye molecular probe bound to cellular nucleic acid of lysed migratory cells. Data was collected by a Fluorescent ELISA reader at 480/520nm and expressed as Relative Fluorescence Units (RFU).

FIGs. 11a-b are graphs depicting effect of the synthetic peptides on MVECs migration. The graphs are illustrating the migration of MVECs (Figure 11a) and migration activation of MVECs (Figure 11b) induced by LT, SP, YR, TR, VL, QF and FGF at concentrations of 1 and 10 ng/ml, following 5 hours of incubation in migration chamber. Data was obtained by measuring the fluorescent enhancement of the CyQuant GR dye molecular probe bound to cellular nucleic acid of lysed migratory

cells. Data was collected by a Fluorescent ELISA reader at 480/520nm and expressed as Relative Fluorescence Units (RFU).

FIG. 12 is a graph depicting the effect of synthetic peptides on arterial ring sprouting. The graph presents the proliferation of ECs in arterial rings induced by four synthetic peptides (i.e., QF, YR, LT and VL) at concentrations 1, 10, 100 and 1000 ng/ml following 7 days incubation in DMEM containing 10 % FCS (37 °C with 5 % CO_2). Data was obtained by estimation of cell proliferation by an XTT assay (O.D. 450 nm).

FIGs. 13a-j are photomicrographs depicting the effect of the peptides on cells tube formation. Figures 13a-e demonstrate MVEC tube formation induced by 8 hours incubation of (Figure 13b) VEGF (Figure 13c) YR, (Figure 13d) QF, (Figure 13e) VL, as compared to untreated control (Figure 13a). Photos were taken after 8 incubation. Figures 13f-j demonstrate EC tube formation induced by 20 hours incubation of (Figure 13g) FGF (Figure 13h) YR, (Figure 13i) QF, (Figure 13j) VL, as compared to untreated control (Figure 13f).

FIGs. 14a-e are bar graphs depicting the effect of synthetic peptides on gene expression in MVECs. The figures demonstrate (Figure 14a) VEGF-A, (Figure 14b) VEGF-C, (Figure 14c) FLT-1, (Figure 14d) KDR and (Figure 14e) HIF-1α, gene expression, 1.5 and 6 hours following addition of synthetic peptides (LT, QF, SP, TR, YR and VL at concentration of 1 ng/ml and VEGF at concentration of 10 ng/ml). Results were measured by real-time PCR and presented as genes expression of treated cells as compared to untreated control.

FIG. 15 is a bar graph demonstrating the intensity of synthetic peptides binding to ECs exposed to the effect of hypoxia treatment. Flow cytometry analysis was effected on the binding of 6 μg FITC labeled synthetic peptide (LT, QF, SP, TR, VL and YR) to 10^5 untreated ECs and ECs after hypoxia. The bars compare mean fluorescence (488nm) obtained after 2 hours incubation of the FITC labeled synthetic peptides with ECs. Data was collected using FACS.

FIGs. 16a-b are graphs depicting the intensity of synthetic peptides binding to ECs exposed to the effect of hypoxia. The graphs present flow cytometry analysis of 6μg FITC labeled peptide (Figure 16a) SP or (Figure 16b) LT to 100000 ECs (red line), ECs after hypoxia (green line) or isotype control (black line) following 2 hours incubation.

FIGs. 17a-f are graphs depicting the effect of synthetic peptides on cells proliferation. Figures 17a-c are illustrating the proliferation of HUVECs induced by (Figure 17a) LT (Figure 17b) SP, or (Figure 17c) QF at concentrations of 0.01, 1, 10, and 100 ng/ml, following 24 hours incubation in EBM-2. Figures 10d-f are illustrating the proliferation of MVECs induced by (Figure 17d) LT (Figure 17e) SP, or (Figure 17f) QF at concentrations of 0.01, 1, 10, and 100 ng/ml following 24 hours incubation in EBM-MV. The plots compare the proliferation of cells under normal conditions (control), under hypoxic conditions or after hypoxic conditions. Data was obtained by measuring radioactive thymidine uptake into cells in a scintillation β counter by cpm/min in the last 6 hours of incubation.

FIGs. 18a-e are photomicrographs depicting the effect of SP on HUVEC and MVEC tube formation under hypoxic conditions. Figures 18a-c demonstrate EC tube formation under hypoxia, induced by 18 hours incubation with (Figure 18b) FGF, or (Figure 18c) SP as compared to control (Figure 18a). Figures 18d-e demonstrate MVEC tube formation under hypoxia, induced by 18 hours incubation with (Figure 18d) FGF, or (Figure 18e) SP. Photos were taken after 18 hours incubation.

FIGs. 19a-e are photomicrographs depicting the effect of the peptides on vascularization of mouse ears. The figures demonstrate the vascularization as induced by subcutaneous injection of (Figure 19a) VEGF (100 ng/mouse ear), (Figure 19b) LT, (Figure 19c) YR, (Figure 19d) QF, or (Figure 19e) SP at a concentration of 10 μg diluted in 10 μl PBS to a mouse ear as compared with 10 μl PBS injected to the other ear (control). Photos were taken 2 days following injection.

FIGs. 20a-b are photomicrographs depicting the effect of TR peptide on vascularization of mouse ear. The figures are histology sections demonstrating vascularization and neo-vascularization induced by 10 μg TR (Figure 20b) as compared to control (Figure 20a). Note, injection of TR peptide reveals large blood vessel formation and neovascularization as demonstrated by capillary blood vessels with single erythrocyte cell.

FIG. 21 is a bar graph depicting median flux of ischemic hind limb/control in a rat ischemic hind limb model. The figure presents the ischemic hind limb blood flow measured at days 4, 7, 9 and 13. Results are the mean of 4, 7, 9, and 13 days after peptide inoculation after 600 μg of VL, LT, QF, TR, SP, YR, FGF or PBS injection to the ischemic leg as compared to the other leg. Results are expressed as

OP/control x100 median flux (Percent median flux of the operated leg versus non operated control leg). Data was obtained at days 4, 7, 9, 13 using a Laser Doppler Blood Flow analyzer.

FIG. 22 is an amino acid sequence alignment of the peptides of the present invention uncovering a conserved sequence motif which is shared by mouse VEGF-B (Swiss-Prot Accession: VEGB_MOUSE).

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is of peptides, which can be used for promoting tissue angiogenesis. Specifically, the present invention can be used to treat angiogenesis-dependent diseases, such as ischemic vascular diseases.

The principles and operation of the present invention may be better understood with reference to the drawings and accompanying descriptions.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

Angiogenesis is the process of generating new capillary blood vessels involving aninterplay between cells and soluble factors (1). The process is characterized by the migration of activated endothelial cells which proliferate to form new vessels, which are surrounded by layers of periendothelial cells, including pericytes for small blood vessels and smooth muscle cells for large blood vessels.

Angiogenesis-dependent diseases are a consequent of an imbalanced angiogenic process resulting in excessive new blood vessels or insufficient blood vessels. Insufficient angiogenesis is related to a large number of diseases and conditions such as coronary artery diseases and delayed wound healing.

Therapeutic angiogenesis is aimed at stimulating new blood vessel growth. The concept of such a therapy is based on the premise that the inherent potential of vascularization in a vascular tissue can be utilized to promote the development of new blood vessels under the influence of the appropriate angiogenic molecules.

While reducing the present invention to practice, the present inventors used a 12-mer phage display peptide library to uncover peptides which are able to bind the cell-surface of endothelial cells incubated under normoxic or hypoxic conditions.

As is illustrated in the Examples section, which follows, the peptides of the present invention triggered angiogenic reactions including, endothelial cell-proliferation migration, aortic ring sprouting, tube formation and *in-vivo* vascularization. These findings suggest that the peptides of the present invention can be used in treatment of various angiogenesis-dependent diseases, such as ischemic-vascular diseases. Furthermore, characterizing the nature of endothelial cell signaling by these peptides will provide the basis for the development of targeted angiogenic therapy for diseases such as cardiovascular disease.

Thus, according to one aspect of the present invention there is provided a peptide including an amino acid sequence as set forth in SEQ ID NO: 13, the peptide is at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 31, at least 32, at least 33, at least 34, at least 35, at least 36, at least 37, at least 38, at least 39, at least 40, at least 41, at least 42, at least 43, at least 44, at least 45, at least 46, at least 47, at least 48, at least 49 and no more than 50 amino acid residues in length.

As is shown in Example 5 of the Examples section, the peptides of this aspect of the present invention share a conserved amino acid sequence (SEQ ID NO: 13) with mammalian vascular endothelial growth factor B (VEGF-B, SwissProt/TrEMBL Accession. VEGB_MOUSE), thereby substantiating the angiogenic function attributed to the peptides of this aspect of the present invention.

According to one preferred embodiment of this aspect of the present invention the peptide is as set forth in SEQ ID NO: 2, 6 or 12.

According to another preferred embodiment of this aspect of the present invention the amino acid sequence is as set forth in SEQ ID NO: 2, 6 or 12.

According to another aspect of the present invention there is provided a peptide including an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10 and 12, the peptide being no more than 50 amino acid residues in length.

According to yet another aspect of the present invention there is provided a peptide including an amino acid sequence as set forth in SEQ ID NO: 14, the peptide is at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 31, at least 32, at least 33, at least 34, at least 35, at least 36, at least 37, at least 38, at least 39, at least 40, at least 41, at least 42, at least 43, at least 44, at least 45, at least 46, at least 47, at least 48, at least 49 and no more than 50 amino acid residues in length.

The term "peptide" as used herein encompasses native peptides (either degradation products, synthetically synthesized peptides or recombinant peptides) and peptidomimetics (typically, synthetically synthesized peptides), as well as peptoids and semipeptoids which are peptide analogs, which may have, for example, modifications rendering the peptides more stable while in a body or more capable of penetrating into cells. Such modifications include, but are not limited to N terminus modification, C terminus modification, peptide bond modification, including, but not limited to, CH2-NH, CH2-S, CH2-S=O, O=C-NH, CH2-O, CH2-CH2, S=C-NH, CH=CH or CF=CH, backbone modifications, and residue modification. Methods for preparing peptidomimetic compounds are well known in the art and are specified, for example, in Quantitative Drug Design, C.A. Ramsden Gd., Chapter 17.2, F. Choplin Pergamon Press (1992), which is incorporated by reference as if fully set forth herein.

Further details in this respect are provided hereinunder. Peptide bonds (-CO-NH-) within the peptide may be substituted, for example, by N-methylated bonds (-N(CH3)-CO-), ester bonds (-C(R)H-C-O-O-C(R)-N-), ketomethylen bonds (-CO-CH2-), α-aza bonds (-NH-N(R)-CO-), wherein R is any alkyl, e.g., methyl, carba bonds (-CH2-NH-), hydroxyethylene bonds (-CH(OH)-CH2-), thioamide bonds (-CS-NH-), olefinic double bonds (-CH=CH-), retro amide bonds (-NH-CO-), peptide derivatives (-N(R)-CH2-CO-), wherein R is the "normal" side chain, naturally presented on the carbon atom.

These modifications can occur at any of the bonds along the peptide chain and even at several (2-3) at the same time.

Natural aromatic amino acids, Trp, Tyr and Phe, may be substituted for synthetic non-natural acid such as Phenylglycine, TIC, naphthylelanine (Nol), ring-methylated derivatives of Phe, halogenated derivatives of Phe or o-methyl-Tyr.

In addition to the above, the peptides of the present invention may also include one or more modified amino acids or one or more non-amino acid monomers (e.g. fatty acids, complex carbohydrates etc).

As used herein in the specification and in the claims section below the term "amino acid" or "amino acids" is understood to include the 20 naturally occurring amino acids; those amino acids often modified post-translationally *in vivo*, including, for example, hydroxyproline, phosphoserine and phosphothreonine; and other unusual amino acids including, but not limited to, 2-aminoadipic acid, hydroxylysine, isodesmosine, nor-valine, nor-leucine and ornithine. Furthermore, the term "amino acid" includes both D- and L-amino acids.

Tables 1 and 2 below list naturally occurring amino acids (Table 1) and non-conventional or modified amino acids (e.g., synthetic, Table 2) which can be used with the present invention.

Table 1

Amino Acid	*Three-Letter Abbreviation*	*One-letter Symbol*
alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic Acid	Glu	E
glycine	Gly	G
Histidine	His	H
isoleucine	Iie	I
leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
tryptophan	Trp	W
tyrosine	Tyr	Y
Valine	Val	V
Any amino acid as above	Xaa	X

Table 2

Non-conventional amino acid	*Code*	*Non-conventional amino acid*	*Code*
α-aminobutyric acid	Abu	L-N-methylalanine	Nmala
α-amino-α-methylbutyrate	Mgabu	L-N-methylarginine	Nmarg
aminocyclopropane-	Cpro	L-N-methylasparagine	Nmasn
carboxylate		L-N-methylaspartic acid	Nmasp
aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
aminonorbornyl-	Norb	L-N-methylglutamine	Nmgin
carboxylate		L-N-methylglutamic acid	Nmglu
cyclohexylalanine	Chexa	L-N-methylhistidine	Nmhis
cyclopentylalanine	Cpen	L-N-methylisolleucine	Nmile
D-alanine	Dal	L-N-methylleucine	Nmleu
D-arginine	Darg	L-N-methyllysine	Nmlys
D-aspartic acid	Dasp	L-N-methylmethionine	Nmmet
D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
D-isoleucine	Dile	L-N-methylproline	Nmpro
D-leucine	Dleu	L-N-methylserine	Nmser
D-lysine	Dlys	L-N-methylthreonine	Nmthr
D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
D-phenylalanine	Dphe	L-N-methylvaline	Nmval
D-proline	Dpro	L-N-methylethylglycine	Nmetg
D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
D-threonine	Dthr	L-norleucine	Nle
D-tryptophan	Dtrp	L-norvaline	Nva
D-tyrosine	Dtyr	α-methyl-aminoisobutyrate	Maib
D-valine	Dval	α-methyl-γ-aminobutyrate	Mgabu
D-α-methylalanine	Dmala	α-methylcyclohexylalanine	Mchexa
D-α-methylarginine	Dmarg	α-methylcyclopentylalanine	Mcpen
D-α-methylasparagine	Dmasn	α-methyl-α-napthylalanine	Manap
D-α-methylaspartate	Dmasp	α- methylpenicillamine	Mpen
D-α-methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
D-α-methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
D-α-methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
D-α-methylisoleucine	Dmile	N- amino-α-methylbutyrate	Nmaabu
D-α-methylleucine	Dmleu	α-napthylalanine	Anap
D-α-methyllysine	Dmlys	N-benzylglycine	Nphe
D-α-methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
D-α-methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
D-α-methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
D-α-methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
D-α-methylserine	Dmser	N-cyclobutylglycine	Ncbut
D-α-methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
D-α-methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
D-α-methyltyrosine	Dmty	N-cyclodecylglycine	Ncdec
D-α-methylvaline	Dmval	N-cyclododeclglycine	Ncdod
D-α-methylalnine	Dnmala	N-cyclooctylglycine	Ncoct

D-α-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
D-α-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
D-α-methylasparatate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
D-α-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
D-N-methylleucine	Dnmleu	N-(3-indolylyethyl) glycine	Nhtrp
D-N-methyllysine	Dnmlys	N-methyl-γ-aminobutyrate	Nmgabu
N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
N-(2-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nva
D-N-methyltyrosine	Dnmtyr	N-methyla-napthylalanine	Nmanap
D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
γ-aminobutyric acid	Gabu	N-(*p*-hydroxyphenyl)glycine	Nhtyr
L-*t*-butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
L-ethylglycine	Etg	penicillamine	Pen
L-homophenylalanine	Hphe	L-α-methylalanine	Mala
L-α-methylarginine	Marg	L-α-methylasparagine	Masn
L-α-methylaspartate	Masp	L-α-methyl-*t*-butylglycine	Mtbug
L-α-methylcysteine	Mcys	L-methylethylglycine	Metg
L-α-methylglutamine	Mgln	L-α-methylglutamate	Mglu
L-α-methylhistidine	Mhis	L-α-methylhomo phenylalanine	Mhphe
L-α-methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
D-N-methylglutamine	Dnmgln	N-(3-guanidinopropyl)glycine	Narg
D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
D-N-methylhistidine	Dnmhis	N-(hydroxyethyl)glycine	Nser
D-N-methylisoleucine	Dnmile	N-(imidazolylethyl)glycine	Nhis
D-N-methylleucine	Dnmleu	N-(3-indolylyethyl)glycine	Nhtrp
D-N-methyllysine	Dnmlys	N-methyl-γ-aminobutyrate	Nmgabu
N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
D-N-methyltyrosine	Dnmtyr	N-methyla-napthylalanine	Nmanap
D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
γ-aminobutyric acid	Gabu	N-(*p*-hydroxyphenyl)glycine	Nhtyr
L-*t*-butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
L-ethylglycine	Etg	penicillamine	Pen
L-homophenylalanine	Hphe	L-α-methylalanine	Mala
L-α-methylarginine	Marg	L-α-methylasparagine	Masn
L-α-methylaspartate	Masp	L-α-methyl-*t*-butylglycine	Mtbug
L-α-methylcysteine	Mcys	L-methylethylglycine	Metg
L-α-methylglutamine	Mgln	L-α-methylglutamate	Mglu
L-α-methylhistidine	Mhis	L-α-methylhomophenylalanine	Mhphe
L-α-methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet

L-α-methylleucine	Mleu	L-α-methyllysine	Mlys
L-α-methylmethionine	Mmet	L-α-methylnorleucine	Mnle
L-α-methylnorvaline	Mnva	L-α-methylornithine	Morn
L-α-methylphenylalanine	Mphe	L-α-methylproline	Mpro
L-α-methylserine	mser	L-α-methylthreonine	Mthr
L-α-methylvaline	Mtrp	L-α-methyltyrosine	Mtyr
L-α-methylleucine	Mval Nnbhm	L-N-methylhomophenylalanine	Nmhphe
N-(N-(2,2-diphenylethyl)		N-(N-(3,3-diphenylpropyl)	
carbamylmethyl-glycine	Nnbhm	carbamylmethyl(1)glycine	Nnbhe
1-carboxy-1-(2,2-diphenyl ethylamino)cyclopropane	Nmbc		

The peptides of the present invention are preferably utilized in a linear form, although it will be appreciated that in cases where cyclization does not severely interfere with peptide characteristics, cyclic forms of the peptide can also be utilized. Cyclic peptides can either be synthesized in a cyclic form or configured so as to assume a cyclic form under desired conditions (e.g., physiological conditions).

It will be appreciated that since one of the main obstacles in using short peptide fragments in therapy is their proteolytic degradation by stereospecific cellular proteases, the peptides of the present invention are preferably synthesized from D-isomers of natural amino acids [i.e., inverso peptide analogues, Tjernberg (1997) J. Biol. Chem. 272:12601-5, Gazit (2002) Curr. Med. Chem. 9:1667-1675].

Additionally, the peptides of the present invention include retro, inverso and retro-inverso analogues thereof. It will be appreciated that complete or extended partial retro-inverso analogues of hormones have generally been found to retain or enhance biological activity. Retro-inversion has also found application in the area of rational design of enzyme inhibitors (see U.S. Pat. No. 6,261,569).

As used herein a "retro peptide" refers to peptides which are made up of L-amino acid residues which are assembled in opposite direction to the native peptide sequence.

Retro-inverso modification of naturally occurring polypeptides involves the synthetic assembly of amino acids with α-carbon stereochemistry opposite to that of the corresponding L-amino acids, i.e., D- or D-allo-amino acids in inverse order to the native peptide sequence. A rerto inverso analogue, thus, has reversed termini and reversed direction of peptide bonds, while essentially maintaining the topology of the side chains as in the native peptide sequence.

It will be appreciated that incorporation of any of the above-mentioned amino acid modifications including conserved changes in amino acid residues of the peptides of the present invention can be effected, as long as the angiogenic function (e.g., endothelial cell proliferation, migration, vascular sprouting, vascularization) of the peptides of the present invention is retained. To test this, any of the angiogenesis assays described hereinbelow and in the Examples section which follows can be effected.

The peptides of present invention can be biochemically synthesized such as by using standard solid phase techniques. These methods include exclusive solid phase synthesis, partial solid phase synthesis methods, fragment condensation, classical solution synthesis. These methods are preferably utilized when the peptide is relatively short (i.e., 10 kDa) and/or when it cannot be produced by recombinant techniques (i.e., not encoded by a nucleic acid sequence) and therefore involves different chemistry.

Solid phase peptide synthesis procedures are well known in the art and further described by John Morrow Stewart and Janis Dillaha Young, Solid Phase Peptide Syntheses (2nd Ed., Pierce Chemical Company, 1984).

Synthetic peptides can be purified by preparative high performance liquid chromatography [Creighton T. (1983) Proteins, structures and molecular principles. WH Freeman and Co. N.Y.] and the composition of which can be confirmed via amino acid sequencing.

Recombinant techniques are preferably used when large amounts of the peptides are required. Such recombinant techniques are described by Bitter et al., (1987) Methods in Enzymol. 153:516-544, Studier et al. (1990) Methods in Enzymol. 185:60-89, Brisson et al. (1984) Nature 310:511-514, Takamatsu et al. (1987) EMBO J. 6:307-311, Coruzzi et al. (1984) EMBO J. 3:1671-1680 and Brogli et al., (1984) Science 224:838-843, Gurley et al. (1986) Mol. Cell. Biol. 6:559-565 and Weissbach & Weissbach, 1988, Methods for Plant Molecular Biology, Academic Press, NY, Section VIII, pp 421-463.

To produce a peptide of the present invention using recombinant technology, a polynucleotide encoding a peptide of the present invention (e.g., SEQ ID NO: 1, 3, 5, 7, 9 or 11) is ligated into a nucleic acid expression construct, which includes the polynucleotide sequence under the transcriptional control of a promoter sequence

suitable for directing constitutive tissue specific or inducible transcription in the host cells, as further described hereinbelow.

Other then containing the necessary elements for the transcription and translation of the inserted coding sequence, the expression construct of the present invention can also include sequences engineered to enhance stability, production, purification, yield or toxicity of the expressed polypeptide. Such a fusion protein can be designed so that the fusion protein can be readily isolated by affinity chromatography; e.g., by immobilization on a column specific for the heterologous protein. Where a cleavage site is engineered between the peptide moiety and the heterologous protein, the peptide can be released from the chromatographic column by treatment with an appropriate enzyme or agent that disrupts the cleavage site [e.g., see Booth et al. (1988) Immunol. Lett. 19:65-70; and Gardella et al., (1990) J. Biol. Chem. 265:15854-15859].

A variety of prokaryotic or eukaryotic cells can be used as host-expression systems to express the peptide coding sequence. These include, but are not limited to, microorganisms, such as bacteria transformed with a recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vector containing the peptide coding sequence; yeast transformed with recombinant yeast expression vectors containing the peptide coding sequence; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors, such as Ti plasmid, containing the peptide coding sequence. Mammalian expression systems can also be used to express the peptides of the present invention. Bacterial systems are preferably used to produce recombinant peptides, according to the present invention, thereby enabling a high production volume at low cost.

Other expression systems such as insects and mammalian host cell systems, which are well known in the art can also be used by the present invention.

In any case, transformed cells are cultured under effective conditions, which allow for the expression of high amounts of recombinant peptides. Effective culture conditions include, but are not limited to, effective media, bioreactor, temperature, pH and oxygen conditions that permit protein production. An effective medium refers to any medium in which a cell is cultured to produce the recombinant peptides of the present invention. Such a medium typically includes an aqueous solution having assimilable

carbon, nitrogen and phosphate sources, and appropriate salts, minerals, metals and other nutrients, such as vitamins. Cells of the present invention can be cultured in conventional fermentation bioreactors, shake flasks, test tubes, microtiter dishes, and petri plates. Culturing can be carried out at a temperature, pH and oxygen content appropriate for a recombinant cell. Such culturing conditions are within the expertise of one of ordinary skill in the art.

Depending on the vector and host system used for production, resultant proteins of the present invention may either remain within the recombinant cell; be secreted into the fermentation medium; be secreted into a space between two cellular membranes, such as the periplasmic space in *E. coli*; or be retained on the outer surface of a cell or viral membrane.

Following a certain time in culture, recovery of the recombinant protein is effected. The phrase "recovering the recombinant protein refers to collecting the whole fermentation medium containing the protein and need not imply additional steps of separation or purification. Proteins of the present invention can be purified using a variety of standard protein purification techniques, such as, but not limited to, affinity chromatography, ion exchange chromatography, filtration, electrophoresis, hydrophobic interaction chromatography, gel filtration chromatography, reverse phase chromatography, concanavalin A chromatography, chromatofocusing and differential solubilization.

The peptides of the present invention are preferably retrieved in "substantially pure" form. As used herein, "substantially pure" refers to a purity that allows for the effective use of the protein in the diverse applications, described herein.

As mentioned hereinabove, the peptides of the present invention can be used to promote angiogenesis (i.e., vascularization) in a tissue of a subject even under hypoxic conditions.

As used herein the term "subject" refers to a mammal such as a canine, a feline, a bovine, a porcine, an equine. Preferably, the subject of the present invention is human.

The subject of this aspect of the present invention may suffer from an angiogenesis-dependent disease or disorder. Examples include, but are not limited to delayed wound-healing, delayed ulcer healing, reproduction associated disorders, arteriosclerosis, myocardial ischemia, peripheral ischemia, cerebral ischemia,

retinopathy, remodeling disorder, von Hippel-Lindau syndrome, diabetes and hereditary hemorrhagic telengiectasia.

The peptides of the present invention can be provided to an individual *per se*, or as part of a pharmaceutical composition where one peptide or more is mixed with a pharmaceutically acceptable carrier.

As used herein a "pharmaceutical composition" refers to a preparation of one or more of the active ingredients described herein with other chemical components such as physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

Herein the term "active ingredient" refers to the peptide preparation, which is accountable for the biological effect.

Hereinafter, the phrases "physiologically acceptable carrier" and "pharmaceutically acceptable carrier" which may be interchangeably used refer to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound. An adjuvant is included under these phrases. One of the ingredients included in the pharmaceutically acceptable carrier can be for example polyethylene glycol (PEG), a biocompatible polymer with a wide range of solubility in both organic and aqueous media (Mutter et al. (1979).

Herein the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition, which is incorporated herein by reference.

Suitable routes of administration may, for example, include oral, rectal, transmucosal, especially transnasal, intestinal or parenteral delivery, including intramuscular, subcutaneous and intramedullary injections as well as intrathecal, direct intraventricular, intravenous, inrtaperitoneal, intranasal, or intraocular injections.

Alternately, one may administer a preparation in a local rather than systemic manner, for example, via injection of the preparation directly into a specific region of a patient's body.

Pharmaceutical compositions of the present invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active ingredients into preparations which, can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the active ingredients of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological salt buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for oral ingestion by a patient. Pharmacological preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carbomethylcellulose; and/or physiologically acceptable polymers such as polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical compositions, which can be used orally, include push-fit capsules made of gelatin as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules may contain the active ingredients in admixture with filler such as lactose, binders such as starches, lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active ingredients may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for the chosen route of administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by nasal inhalation, the active ingredients for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from a pressurized pack or a nebulizer with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane or carbon dioxide. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in a dispenser may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The preparations described herein may be formulated for parenteral administration, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multidose containers with optionally, an added preservative. The compositions may be suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical compositions for parenteral administration include aqueous solutions of the active preparation in water-soluble form. Additionally, suspensions

of the active ingredients may be prepared as appropriate oily or water based injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acids esters such as ethyl oleate, triglycerides or liposomes. Aqueous injection suspensions may contain substances, which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the active ingredients to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water based solution, before use.

The preparation of the present invention may also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

Pharmaceutical compositions suitable for use in context of the present invention include compositions wherein the active ingredients are contained in an amount effective to achieve the intended purpose. More specifically, a therapeutically effective amount means an amount of active ingredients effective to prevent, alleviate or ameliorate symptoms of disease or prolong the survival of the subject being treated.

Determination of a therapeutically effective amount is well within the capability of those skilled in the art.

For any preparation used in the methods of the invention, the therapeutically effective amount or dose can be estimated initially from in vitro assays. For example, a dose can be formulated in animal models and such information can be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the active ingredients described herein can be determined by standard pharmaceutical procedures *in vitro*, in cell cultures or experimental animals. The data obtained from these *in vitro* and cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. [See e.g., Fingl, et al., (1975) "The Pharmacological Basis of Therapeutics", Ch. 1 p.1].

Depending on the severity and responsiveness of the condition to be treated, dosing can be of a single or a plurality of administrations, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the disease state is achieved.

The amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

Compositions including the preparation of the present invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

Compositions of the present invention may, if desired, be presented in a pack or dispenser device, such as an FDA approved kit, which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accommodated by a notice associated with the container in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or human or veterinary administration. Such notice, for example, may be of labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert.

It will be appreciated that the peptides of the present invention can also be expressed from a nucleic acid construct administered to the subject employing any suitable mode of administration, described hereinabove (i.e., in-vivo gene therapy). Alternatively, the nucleic acid construct is introduced into a suitable cell via an appropriate gene delivery vehicle/method (transfection, transduction, homologous recombination, etc.) and an expression system as needed and then the modified cells are expanded in culture and returned to the individual (i.e., ex-vivo gene therapy). However, to enable secretion of the peptides of the present invention the polynucleotides encoding thereof (e.g., SEQ ID NO: 1, 3, 5, 7, 9 or 11) preferably further include a polynucleotide sequence which encodes an inframe signal peptide (e.g., such as the signal peptide of human VEGF-B Swiss-Prot/TrEMBL Accession VEGB_HUMAN).

To enable cellular expression of the peptides of the present invention, the nucleic acid construct of the present invention further includes at least one cis acting regulatory element. As used herein, the phrase "cis acting regulatory element" refers to a polynucleotide sequence, preferably a promoter, which binds a trans acting regulator and regulates the transcription of a coding sequence located downstream thereto.

Any available promoter can be used by the present methodology. In a preferred embodiment of the present invention, the promoter utilized by the nucleic acid construct of the present invention is active in the specific cell population transformed. Examples of cell type-specific and/or tissue-specific promoters include promoters such as albumin that is liver specific [Pinkert et al., (1987) Genes Dev. 1:268-277], lymphoid specific promoters [Calame et al., (1988) Adv. Immunol. 43:235-275]; in particular promoters of T-cell receptors [Winoto et al., (1989) EMBO J. 8:729-733] and immunoglobulins; [Banerji et al. (1983) Cell 33729-740], neuron-specific promoters such as the neurofilament promoter [Byrne et al. (1989) Proc. Natl. Acad. Sci. USA 86:5473-5477], pancreas-specific promoters [Edlunch et al. (1985) Science 230:912-916] or mammary gland-specific promoters such as the milk whey promoter (U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). The nucleic acid construct of the present invention can further include an enhancer, which can be adjacent or distant to the promoter sequence and can function in up regulating the transcription therefrom.

The nucleic acid construct of the present methodology preferably further includes an appropriate selectable marker and/or an origin of replication. Preferably, the construct utilized is a shuttle vector, which can propagate both in *E. coli* (wherein the construct comprises an appropriate selectable marker and origin of replication) and be compatible for propagation in cells, or integration in a gene and a tissue of choice. The construct according to the present invention can be, for example, a plasmid, a bacmid, a phagemid, a cosmid, a phage, a virus or an artificial chromosome.

Currently preferred in vivo nucleic acid transfer techniques include transfection with viral or non-viral constructs, such as adenovirus, lentivirus, Herpes simplex I virus, or adeno-associated virus (AAV) and lipid-based systems. Useful lipids for lipid-mediated transfer of the gene are, for example, DOTMA, DOPE, and

DC-Chol [Tonkinson et al., Cancer Investigation, 14(1): 54-65 (1996)]. The most preferred constructs for use in gene therapy are viruses, most preferably adenoviruses, AAV, lentiviruses, or retroviruses. A viral construct such as a retroviral construct includes at least one transcriptional promoter/enhancer or locus-defining element(s), or other elements that control gene expression by other means such as alternate splicing, nuclear RNA export, or post-translational modification of messenger. Such vector constructs also include a packaging signal, long terminal repeats (LTRs) or portions thereof, and positive and negative strand primer binding sites appropriate to the virus used, unless it is already present in the viral construct. In addition, such a construct typically includes a signal sequence for secretion of the peptide or antibody from a host cell in which it is placed. Preferably the signal sequence for this purpose is a mammalian signal sequence. Optionally, the construct may also include a signal that directs polyadenylation, as well as one or more restriction sites and a translation termination sequence. By way of example, such constructs will typically include a 5' LTR, a tRNA binding site, a packaging signal, an origin of second-strand DNA synthesis, and a 3' LTR or a portion thereof. Other vectors can be used that are non-viral, such as cationic lipids, polylysine, and dendrimers.

Due to their selective binding to endothelial cells, the peptides of the present invention can be used to target agents fused thereto to ECs and thus can also be used for treating, i.e., curing, preventing or substantially reducing symptoms of angiogenesis-dependent diseases which are characterized by hyper-vascularization. For example, such fusions which include drugs can be used to inhibit tumor growth by destruction of the tumor vasculature.

Examples of drugs which can be included in such compositions include, but are not limited to, toxins, such as enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof [e.g., diphteria toxin, exotoxin A chain of Pseudomonas aeruginosa, ricin A chain, abrin A chain, modeccin A chain, α-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes], radioisotopes (e.g., ^{125}I, ^{131}I, ^{90}Y, ^{212}Bi and ^{198}Re) and chemotherapeutic agents (e.g., alkylating agents, folic acid antagonists, anti-metabolites of nucleic acid metabolism, antibiotics, pyrimidine analogs, 5-

fluorouracil, cisplatin, purine nucleosides, amines, amino acids, triazol nucleosides, or corticosteroids. Specific examples include, Adriamycin, Doxorubicin, 5-Fluorouracil, Cytosine arabinoside (i.e., Ara-C), Cyclophosphamide, Thiotepa, Busulfan, Cytoxin, Taxol, Toxotere, Methotrexate, Cisplatin, Melphalan, Vinblastine, Bleomycin, Etoposide, Ifosfamide, Mitomycin C, Mitoxantrone, Vincreistine, Vinorelbine, Carboplatin, Teniposide, Daunomycin, Carminomycin, Aminopterin, Dactinomycin, Mitomycins, Esperamicins (see U.S. Pat. No. 4,675,187), Melphalan, and other related nitrogen mustards. Also included in this definition are hormonal agents that act to regulate or inhibit hormone action on tumors, such as tamoxifen and onapristone.

Fusions between the peptides of the present invention and drugs can be generated using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutareldehyde), bisazido compounds (such as bis-(p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin fusion can be prepared as described in Vitetta et al., Science, 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the peptide. See WO94/11026; U.S. Pat. No. 6,426,400; Laske, D. W., Youle, R. J., and Oldfield, E. H. (1997) Tumor regression with regional distribution of the targeted toxin TF-CRM107 in patients with malignant brain tumors. Nature Medicine 3:1362-1368.

The ability of the peptides of the present invention to promote angiogenesis in-vivo see Example 4, suggests that antibodies directed there against, which neutralize their activity (i.e., neutralizing antibodies) can be used as potent anti-angiogenic drugs, which may be used as important therapeutic tools for the treatment of angiogenesis-dependent diseases which are characterized by hyper-vascularization (e.g., cancer. Further Examples are provided hereinbelow).

The term "antibody" refers to intact antibody molecules as well as functional fragments thereof, such as Fab, $F(ab')_2$, and Fv that are capable of binding to antigen

presenting cells. These functional antibody fragments are defined as follows: (i) Fab, the fragment which contains a monovalent antigen-binding fragment of an antibody molecule, can be produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain; (ii) Fab', the fragment of an antibody molecule that can be obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain; two Fab' fragments are obtained per antibody molecule; (iii) $(Fab')_2$, the fragment of the antibody that can be obtained by treating whole antibody with the enzyme pepsin without subsequent reduction; $F(ab')_2$ is a dimer of two Fab' fragments held together by two disulfide bonds; (iv) Fv, defined as a genetically engineered fragment containing the variable region of the light chain and the variable region of the heavy chain expressed as two chains; and (v) Single chain antibody ("SCA"), a genetically engineered molecule containing the variable region of the light chain and the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule.

Methods of making these fragments are known in the art. (See for example, Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York, 1988, incorporated herein by reference).

Methods of generating antibodies (i.e., monoclonal and polyclonal) are well known in the art. Antibodies may be generated via any one of several methods known in the art, which methods can employ induction of in vivo production of antibody molecules, screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed [Orlandi D.R. et al. (1989) Proc. Natl. Acad. Sci. 86:3833-3837, Winter G. et al. (1991) Nature 349:293-299] or generation of monoclonal antibody molecules by continuous cell lines in culture. These include but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the Epstein-Bar-Virus (EBV)-hybridoma technique [Kohler G., et al. (1975) Nature 256:495-497, Kozbor D., et al. (1985) J. Immunol. Methods 81:31-42, Cote R.J. et al. (1983) Proc. Natl. Acad. Sci. 80:2026-2030, Cole S.P. et al. (1984) Mol. Cell. Biol. 62:109-120].

Antibody fragments according to the present invention can be prepared by proteolytic hydrolysis of the antibody or by expression in E. coli or mammalian cells

(e.g. Chinese hamster ovary cell culture or other protein expression systems) of DNA encoding the fragment.

Antibody fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods. For example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted $F(ab')_2$. This fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab' monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab' fragments and an Fc fragment directly. These methods are described, for example, by Goldenberg, U.S. Pat. Nos. 4,036,945 and 4,331,647, and references contained therein, which patents are hereby incorporated by reference in their entirety. See also Porter, R. R., Biochem. J., 73: 119-126, 1959. Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical, or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

Fv fragments comprise an association of V_H and V_L chains. This association may be noncovalent, as described in Inbar et al., Proc. Nat'l Acad. Sci. USA 69:2659-62, 1972. Alternatively, the variable chains can be linked by an intermolecular disulfide bond or cross-linked by chemicals such as glutaraldehyde. Preferably, the Fv fragments comprise V_H and V_L chains connected by a peptide linker. These single-chain antigen binding proteins (ScFv) are prepared by constructing a structural gene comprising DNA sequences encoding the V_H and V_L domains connected by an oligonucleotide. The structural gene is inserted into an expression vector, which is subsequently introduced into a host cell such as E. coli. The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing sFvs are described, for example, by Whitlow and Filpula, Methods, 2: 97-105, 1991; Bird et al., Science 242:423-426, 1988; Pack et al., Bio/Technology 11:1271-77, 1993; and Ladner et al., U.S. Pat. No. 4,946,778, which is hereby incorporated by reference in its entirety. Other methods of obtaining antibody-specific ScFv fragments include screening of single-chain antibody constructs [e.g., library V18 p852; Azriel-Rosenfeld (2004) J. Mol. Biol. 335:177-192] such as described in Current Protocols in Immunology 2002 10/19B.1-10.19B.31

by Wiley and Sons, inc. Recombining germline-derived CDR sequences for creating diverse single-framwork antibody libraries by Soerlind et al Nature 2000.

Another form of an antibody fragment is a peptide coding for a single complementarity-determining region (CDR). CDR peptides ("minimal recognition units") can be obtained by constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction to synthesize the variable region from RNA of antibody-producing cells. See, for example, Larrick and Fry, Methods, 2: 106-10, 1991.

For human applications, the antibodies of the present invention are preferably humanized. Humanized forms of non-human (e.g., murine) antibodies are chimeric molecules of immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab').sub.2 or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues form a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will include at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin [Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol., 2:593-596 (1992)].

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as import residues, which are typically taken from an import variable domain. Humanization can be essentially performed following the method of Winter

and co-workers [Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature 332:323-327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988)], by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such humanized antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

Human antibodies can also be produced using various techniques known in the art, including phage display libraries [Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222:581 (1991)]. The techniques of Cole et al. and Boerner et al. are also available for the preparation of human monoclonal antibodies (Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985) and Boerner et al., J. Immunol., 147(1):86-95 (1991)]. Similarly, human can be made by introducing of human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks et al., Bio/Technology 10, 779-783 (1992); Lonberg et al., Nature 368 856-859 (1994); Morrison, Nature 368 812-13 (1994); Fishwild et al., Nature Biotechnology 14, 845-51 (1996); Neuberger, Nature Biotechnology 14, 826 (1996); Lonberg and Huszar, Intern. Rev. Immunol. 13 65-93 (1995).

It will be appreciated that the antibodies of the present invention can be used to uncover the native protein which the peptides of the present mimic the activity thereof. Biochmeical methods of isolation are well known in the art and may include the attachment of the antibodies of the present invention to solid support (e.g., columns) for the mass screening of biological samples, preferably fluids. For further details see "Current Protocols in Protein Science" Chap. 1 "Strategies of Protein Purification and Characterization" Wiley and Sons Inc. 2003.

A growing body of evidence indicates that angiogenesis is essential to the progression of cancer. In fact, the extent of neovascularity is strongly correlated with metastases in primary breast carcinoma, bladder cancer, prostrate cancer, non-small cell lung cancer, cutaneous melanomas, and uterine cervix carcinoma [Ferrara, N., Breast Cancer Research and Treatment 36: 127-137 (1995)]. Thus, assessing the angiogenic phenotype of tumors will provide a strong indication to disease outcome. Other diseases or conditions which are characterized by hypervascularization or hypovascularization include, but are not limited to, retinal neovascularization, neovascularization in atherosclerotic plaques, hemangiomas, arthritis, and psoriasis, as well as the diseases described hereinabove. See Folkman, J. New England J. of Med. 333:1757-63 (1995).

Thus, the ability of the peptides of the present invention to bind specifically to the cell-surface of endothelial cells, suggests the use thereof as potent detectors of vascularization. This may be important for detecting the presence of, assessing predisposition to, or monitoring progression of an angiogenesis-dependent diseases.

Thus, the present invention also envisages a method of detecting a presence or an absence of endothelial cells in a biological sample:

The method is effected by incubating the biological sample with a peptide of the present invention capable of binding to the cell-surface of endothelial cells and detecting the peptide, to thereby detect the presence or the absence of endothelial cells in the biological sample.

The biological sample utilized for detection is preferably a tissue sample such as a biopsy specimen. Methods of obtaining tissue biopsies from mammals are well known in the art (see http://www.healthatoz.com/healthatoz/Atoz/default.html).

At least one peptide of the present invention is contacted with the biological sample under conditions suitable for complex formation (i.e., buffer, temperature, incubation time etc.); suitable conditions are described in Example 1 of the Examples section.

Peptide-cell complexes within a biological sample can be detected via any one of several methods known in the art, which methods can employ biochemical and/or optical detection schemes.

To facilitate complex detection, the peptides of the present invention are highlighted preferably by a tag or an antibody. It will be appreciated that highlighting can be

effected prior to, concomitant with or following complex formation, depending on the highlighting method. As used herein the term "tag" refers to a molecule, which exhibits a quantifiable activity or characteristic. A tag can be a fluorescent molecule including chemical fluorescers such as fluorescein or polypeptide fluorescers such as the green fluorescent protein (GFP) or related proteins (www.clontech.com). In such case, the tag can be quantified via its fluorescence, which is generated upon the application of a suitable excitatory light. Alternatively, a tag can be an epitope tag, a fairly unique polypeptide sequence to which a specific antibody can bind without substantially cross reacting with other cellular epitopes. Such epitope tags include a Myc tag, a Flag tag, a His tag, a leucine tag, an IgG tag, a streptavidin tag and the like.

It will be appreciated that the peptides of the present invention may also be used as potent detectors of endothelial cells in-vivo. A designed peptide capable of binding endothelial cells, labeled non-radioactively or with a radio-isotope, as is well known in the art can be administered to an individual to diagnose the onset or presence of angiogenesis-dependent disease, discussed hereinabove. The binding of such a labeled peptide after administration to endothelial cells can be detected by in vivo imaging techniques known in the art.

It will be appreciated that such a detection method can also be utilized in an assay for uncovering potential drugs useful in inhibition or promotion of angiogenesis. For example, the present invention may be used for high throughput screening of test compounds (i.e., putative angiogenic molecules). Typically, the peptides of the present invention are radiolabeled, to reduce assay volume. The peptides are allowed to bind endothelial cells prior to, concomitant with or following binding of the test compound. A competition assay is then effected by monitoring displacement of the label by a test compound [Han (1996) J. Am. Chem. Soc. 118:4506-7 and Esler (1996) Chem. 271:8545-8].

Once a putative angiogenic molecule is identified it is further evaluated using angiogenesis assays which are well known in the art. Examples include, but are not limited to, the chick chorioallantoic membrane, rabbit cornea assay, sponge implant models, matrigel and tumor models (see also the assays described in the Examples section which follows).

The peptides and/or antibodies of the present invention can be included in a diagnostic or therapeutic kit. For example, the peptides can be packaged in a one or more containers with appropriate buffers and preservatives and used for diagnosis or for directing therapeutic treatment. Thus, the peptides, for example, can be each mixed in a single container or placed in individual containers. Preferably, the containers include a label. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic.

In addition, other additives such as stabilizers, buffers, blockers and the like may also be added.

The peptides or antibodies of such kits can also be attached to a solid support, such as beads, array substrate (e.g., chips) and the like and used for diagnostic purposes.

Peptides included in kits or immobilized to substrates may be conjugated to a detectable label such as described hereinabove.

The kit can also include instructions for determining if the tested subject is suffering from, or is at risk of developing, a condition, disorder, or disease associated with disregulated angiogenesis.

Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et

al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., Eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

EXAMPLE 1

Identification of novel angiogenesis inducing peptides.

Novel peptides that potentially induce angiogenesis were identified by affinity positive selection (i.e., biopanning) of a random phage display peptide library with

human umbilical vein endothelial cells (HUVECs, ECs), followed by ELISA test of phage positive clones binding to ECs.

MATERIALS AND METHODS

Phage Display Peptide Library:

The Random Phage Display Peptide Library employed in this study was purchased from New England Biolabs, Inc. (MA, USA). The phage display library is based on a combinatorial library of random peptide 12-mers fused to a minor coat protein (pIII) of M13 phage. The displayed 12-mer peptides are expressed at the N-terminus of pIII. The library consists of about $2.7x10^9$ electroporated sequences amplified once to yield 20 copies of each sequence in 10 µl of the supplied phage.

The above-described phage display peptide library was subjected to five rounds of affinity positive selection (biopanning) in screening against differentially treated endothelial cells (ECs): a) ECs without treatment, b) ECs after 3 hours hypoxia, c) ECs after 24 hours hypoxia . Each positive selection was preceded by a negative selection on human Peripheral Blood Lymphocytes (PBL). Each round of biopanning was effected by elution of the bound phages with 0.2 % M glycine- HCl. The unbound phages were incubated on a second EC plate; this procedure was executed three times. Phages of the three eluted phages were pooled for the second round of biopanning and so on. After the fifth round of biopanning 40 individual clones from each group of cells screened were isolated, all together 120 individual clones were obtained.

Screening of positive clones by ELISA:

The binding of positive clones from each group was re-evaluated by ELISA. For this purpose, 96 well plates including ECs under normoxic conditions, ECs after 3, 6 or 24 hours hypoxia, were prepared (20000 cells/well). Plates with human PBL were prepared as controls. Plates were re-hydrated by incubation with PBS + 3 % BSA over night at 4 °C, followed by washing the wells with PBS three times. Phages from each of the 120 clones isolated were dispersed on the ELISA plates in concentrations of 10^{10}, 10^9 or 10^8 phages per well and incubated for two hours at room temperature. Plates were then washed three times with PBS + 0.05 % Tween followed by three washes with DDW. Thereafter, an anti-M13 HRP antibody (Amersham Pharmacia Biotech UK Limited, Buckinghamshire, UK) in a dilution of 1:5000 was added for 2 hours. After washes, a 100µl of tetramethyl benzidine liquid substrate (DAKO TMB

substrate chromogen, DAKO corporation, CA, USA) was added for 15 minutes and the reaction was terminated with the addition of 1M HCl. Plates were read at 450 nm in an ELISA reader (SLT 400ATC SLT LAB instruments).

Human Umbilical Vein Endothelial Cells (HUVEC):

HUVEC (ECs), were isolated by collagenase digestion as previously described [Jaffe et al., J. Clin. Invest., 52(11):2757-64, 1973]. ECs were cultured with M199 supplemented with 20 % FCS, 10000 units penicillin, 10 mg/ml streptomycin sulfate, 10 mg/ml neomycin sulfate (Biological Industries, kibbutz Beit Haemek, Israel), 25 µg/ml EC growth supplement (Biomedical Technologies, MA, USA) and 5 U/ml Heparin (SIGMA, Rehobot, Israel). HUVEC were harvested with Trypsin (0.25 %), EDTA (0.05 %, Biological Industries, kibbutz Beit Haemek, Israel) and incubated on 60 mm petri-dishes coated with 1 % gelatin for 24 hours. After incubation, cells were washed and incubated with M199 supplemented with 10 % FCS. ECs were subjected to four different treatment groups: a) no treatment, b) 3 hours hypoxia, c) 6 hours hypoxia, d) 24 hours hypoxia. Thereafter monolayers were washed with PBS and dried over night. Cells were rehydrated with PBS containing 5 % FCS and 0.1 % sodium azide and kept at 4 °C until the procedure of biopanning.

Hypoxia conditions:

ECs were subjected to hypoxia for 3, 6 or 24 hours with a mix gas of 94 % Nitrogen + 5 % CO_2 + 1 % O_2 in a hypoxia chamber (Billups-Rothenberg, CA, USA).

Statistical and graphical methods:

Statistical analysis was effected by analysis of variance (ANOVA), with appropriate post-hoc tests, generally Dunnett's for comparison to a control or Tukey-Kramer HSD for multiple comparisons. Results are considered statistically significant at $P<0.05$.

RESULTS

EC binding peptides:

A phage display peptide library was subjected to five rounds of affinity positive selections (biopanning) in screening against ECs under physiological conditions and hypoxia. The second step of selection of peptide bound phages was done by ELISA method using ECs and lymphocytes coated plates as controls. As can be seen in Figure 1, fifteen different peptide bound phages at 10^9 (Figure 1a) and 10^{10}

(Figure 1b) per well were screened by ELISA on four different EC preparations (ECs at normal conditions and ECs after 3, 6, and 24 hours of hypoxia).

ANOVA on 10^9 selected phages on the above-described treated cells indicated statistically significant differences ($p<0.05$) between binding of NO phages and binding of certain peptides-bound phages as indicated in Table 1, below and Figure 1a.

ANOVA on 10^{10} selected phages on treated cells indicated statistically significant differences ($p<0.05$) between binding of NO phages and binding of all peptide-bound phages, as indicated in Figure 1b.

Table 1. Peptide-bound phages

Peptide	Cells
SP	EC
SP	H3
TR	EC
TR	H3
TR	H6
TR	H24
VL	EC
VL	H3
VL	H6
VL	H24
YR	EC
YR	H3
YR	H6
YR	H24

EC (ECs at normal conditions), H3 (ECs after 3 hours of hypoxia), H6 (ECs after 6 hours of hypoxia), and H24 (ECs after 24 hours of hypoxia).

Vascularization and re-vascularization are complex biological processes comprised of multiple sequential and simultaneous interacting stages. The initial stage examined, is the ability of the tested peptides for specific binding to the target cells. About 120 of the peptides screened were found to have this ability, and therefore were further examined for other angiogenic characteristics.

EXAMPLE 2

In vitro induced angiogenesis by phage display peptides.

The ability of phage displaying selected peptides to induce angiogenesis was evaluated by induction of ECs proliferation, ECs migration, and sprouting of aortic rings.

MATERIALS AND METHODS

Identification of DNA sequences from selected phages:

DNA from all isolated selected clones was purified by incubation with iodide buffer and ethanol (according to manual instructions, NEB, MA, USA). This rapid procedure produces template of sufficient purity for automated sequencing with dye labeled dideoxynucleotides. The 96 gIII (NEB, MA, USA) sequencing primer was utilized for automated sequencing by the Sequencing Unit of Tel Aviv University.

ECs and hypoxia conditions:

ECs were isolated, cultured, and subjected to hypoxia treatments as described in Example 1 of the Examples section.

EC Proliferation with phage display peptides:

ECs (40000 cells/well) were seeded on 24 well gelatin (1 %)-coated plates cultured with M199 medium supplemented with 20 % FCS, 10000 units penicillin, 10 mg/ml streptomycin sulfate, 10 mg/ml neomycin sulfate (Biological Industries, kibbutz Beit Haemek, Israel), 25 µg/ml endothelial cell growth supplement (Biomedical Technologies, MA, USA) and 5U/ml Heparin (SIGMA, Rehobot, Israel). Following 24 hours, cells were washed with serum free media and incubated with serum free media for another 24 hours. Then, 10^6 phages were added per each well and plates were incubated for 24 hours. Abortive phages (without an exposed peptide), were used as negative controls and were designated NO. 2 µCi/well of thymidine (SIGMA, Rehovot, Israel) were added for the last 6 hours of incubation and then cells were fixed with 10 % TCA for 16 hours at 4 °C. Thereafter, cells were washed with absolute ethanol and incubated for 15 minutes at 37 °C with 300 µl/well of 0.5 M NaOH to lyse cells. Lysates were transferred to a scintillation vial with 2 ml scintillation liquid Ultima Gold (Packard Bioscience, Meriden, USA) and counted in a β counter (Scintillation β counter Packard, A Canberra company, 1600 TR). Results were obtained as cpm/min.

EC migration by phage display peptides:

EC migration was evaluated by the Chemicon QCM 96-well Migration Assay (Chemicon International, CA, USA) according to manufacturer instructions. Briefly, the kit utilizes a membrane with 8μm pores. Migratory cells on the bottom of the insert membrane are dissociated from the membrane when incubated with cell detachment buffer provided by the kit. These cells are subsequently lysed and detected by a molecular probe CyQuant GR dye. The green fluorescent dye exhibits fluorescent enhancement when bound to cellular nucleic acid. For the migration assay, ECs from passage 3 were incubated on gelatin-coated plates in M199 medium free serum for 24 hours. Following trypsinization, $2 \cdot 10^4$ ECs were incubated in each of the 96 wells of the migration chamber. 10^5 or 10^6 phages bound peptides were added to the feeder tray for executing the above-described chemoattractant assay so peptides are not in touch with cells and they play a role of chemoattractants.

For activation migration assays, 10^5 or 10^6 phages bound peptides were incubated with ECs in the migration chamber. Incubation time in both assays was 5 hours. Phage NO (unmodified M13 phages) served as negative control. Results were determined by a fluorescent ELISA reader at 480/520nm (Fluostar BMG Lab Teck).

Aortic ring formation with phage display peptides:

Human mammary or radial artery was removed of adventitia and cut into 1 mm long rings. The bottom of each well of sterile 96-well plate was coated with 20 μg of fibronectin (Biological Industries, Kibbutz Beit Ha Hemek, Israel) and the rings were positioned in the center of each well containing 150 ml of Dulbecco's modified Eagle's medium (DMEM, Biological Industries, Kibbutz Beit Ha Hemek, Israel) containing 10 % FCS. 10^6 selected cloned phages were added to each well. Unmodified M13 phages (NO) were used as negative controls. Plates were incubated at 37 °C with 5 % CO_2 for 7 days. Arterial rings were removed and the extent of cell proliferation was estimated by an XTT assay (Biological Industries, Kibbutz Beit Ha Hemek, Israel) according to manufacturer instructions.

RESULTS

Identification of angiogenesis inducing peptides:

Among positive selected phage screened for EC binding, many were found to have identical sequences. Table 2 demonstrates the number of identical DNA sequences obtained from the positive selected phages of about 120 sequences obtained

from screening ECs (ECs at normal conditions), H3 (ECs after 3 hours of hypoxia), and H24 (ECs after 24 hours of hypoxia).

Table 2. Number of identical sequences obtained from positive selected phages

Cells	EC	H3	H24
VL	22	-	10
LP	2	29	9
TR	2	-	2
ST	2	-	-
QF	4	-	-
NS	2	-	-
SP	-	3	2
YR	-	2	-
LT	-	-	4
HR	-	-	3
HY	-	-	2
TP	-	-	-
NR	-	-	-
SA	-	-	-

EC (ECs at normal conditions), H3 (ECs after 3 hours of hypoxia), and H24 (ECs after 24 hours of hypoxia).

Six individual clones were chosen and their ability to induce ECs proliferation and migration was assayed. Table 3 shows the DNA and protein sequences of these six chosen peptides.

Table 3. Selected peptides

Peptide ID	DNA sequence	SEQ ID NO:	Amino acid sequence	SEQ ID NO:
VL	GTTCCGTGGATGGAGCCGGCTTATCAGAGGTTTCTG	1	VPWMEPAYQRFL	2
LT	CTGCTTGCGGATACGACGCATCATAGGCCGTGGACT	3	LLADTTHHRPWT	4
QF	CAGCCTTGGTTGGAGCAGGCTTATTATAGTACGTTT	5	QPWLEQAYYSTF	6
SP	TCTGCGCATGGGACGTCTACTGGTGTTCCGTGGCCG	7	SAHGTSTGVPWP	8
YR	TATCCGCATATTGATTCGCTTGGTCATTGGCGGCGG	9	YPHIDSLGHWRR	10
TR	ACTTTGCCGTGGCTGGAGGAGTCTTATTGGCGTCCT	11	TLPWLEESYWRP	12

EC induced proliferation by selected peptide presenting phages:

Angiogenic activity of phage bound selected peptides was initially tested by ECs proliferation assays. Figure 2 demonstrates increased proliferation induced by the peptide presenting phages (10^6 phages) as compared to unmodified phages (NO).

EC induced migration by selected peptide presenting phages:

The ability of phage display selected peptides to induce migration of ECs was tested in migration chambers. Peptide presenting phages were incubated with ECs at concentrations of 10^5 (Figure 3a) and 10^6 (Figure 3b) phages. Two of the tested peptides (QF and LT) induced migration of the activated ECs, in the two concentrations tested (Figure 3a and 3b). Placing the phage display peptides at two different concentrations on the feeder tray revealed the ability of YR (of the six selected phage display peptides) to induce migration as chemoattractants at 10^5 phages per well (Figure 4a). 10^6 phages bound to QF, SP, TR and LT induced migration as chemoattractants (Figure 4b).

Aortic ring sprouting by peptide presenting phages:

Aortic rings were tested for sprouting in the presence of phage bound selected peptides. Figure 5 demonstrates proliferation of cells originating from the aortic rings, induced by peptide presenting phages. ANOVA analysis comparing the different phage display peptides indicated an overall clear difference between them (P=0.0003), indicating differences in proliferation of cells derived from the aortic rings, as illustrated in Figure 5. Post-hoc tests indicated statistically significant differences between peptide VL and the S24 empty phage control.

The induction of angiogenesis comprises multiple sequential and simultaneous interacting stages. Following the stage of binding ECs, angiogenic peptides should be capable of inducing proliferation and migration of these cells, as well as tube formation and other processes involved in creating vascular structures. Indeed, in all features tested, several of the peptide presenting phages showed significant effect over control phages, indicating angiogenic effect of their presented peptides.

EXAMPLE 3

In vitro induced angiogenesis by synthetic peptide.

Selected peptides were synthesized and their ability to induce angiogenesis was re-evaluated by induction of ECs proliferation, ECs migration and sprouting of aortic rings.

MATERIALS AND METHODS

Peptides synthesis:

Peptides were synthesized by SynPep (Dublin, CA, USA). HPLC purity analysis showed that the purity of each synthetic peptide was higher than 97 %. All the

peptides were dissolved in water excluding QF that was dissolved in 50 % water + 50 % acetonitrile.

Fluorescein labeling of synthetic peptides:

Fluorescein Isothiocyanate (FITC, Pierce, Rockford, IL) is an amino reactive probe that reacts in alkaline environment with primary amines to form a stable fluorescent derivative. 12.5µl of FITC (10 mg/ml) was added per 1 mg of peptide diluted in 0.5M bicarbonate buffer (pH 9.5) and agitated in the dark for 2 hours. 0.1 ml of 1.5M hydroxilamine was then added per 1 ml of reaction mixture and agitated for additional 30 minutes at room temperature. Unbound FITC was removed by dialysis.

FACS analysis of peptide binding to ECs:

ECs cultured with M199 + 10 % FCS or ECs exposed to 24 hours hypoxia were trypsinated and counted. 100000 cells were suspended in PBS + 5 % FCS + 0.1 % Na azide. Cells were stained with 1-6 µg labeled peptides for 2 hours, on ice, in dark, and then washed twice with PBS. Samples were analyzed by Fluorescence Activated Cell Sorter (FACScan Beckton Dickinson, CA, USA).

EC proliferation with synthetic peptides:

ECs were incubated with EBM-2 medium with supplements (Cambrex BioWhittaker Cell Biology Products, USA). After incubation, cells were harvested with Trypsin (0.25 %) EDTA (0.05 %) (Biological Industries, kibbutz Beit Haemek, Israel) and re-incubated on 25 cm^2 flasks. ECs from passage 3 were used for proliferation experiments.

ECs (12000 cells/well) were seeded on 24 well in EBM-2 medium with supplements. After 24 hours incubation, cells were exposed to 24 hours starvation in medium free supplements. Synthetic peptides (SP and LT, TR and VL) were added in concentrations of 0.05, 0.1, 1, 10 and 100 ng/ml for 24 hours. 2 µCi/well of thymidine (SIGMA, Rehovot, Israel) were added for 6 hours and then plates were washed 3 times with PBS. Plates were incubated for 15 minutes at 37 °C with 300 µl/well of 0.5M NaOH. Then, lysated cells were transferred to a scintillation vial with 2 ml scintillation liquid Ultima Gold (Packard Bioscience, Meriden, USA) and counted in a β counter (Scintillation β Counter Packard A Camberra Company 1600 TR). Results were obtained as cpm/minute.

Dermal Microvascular endothelial cells (MVECs) proliferation with synthetic peptides:

MVECs were incubated and exposed to synthetic peptides (LT, SP, and YR) as described above for HUVECs, but the MVECs were seeded in EBM-MV medium with supplements (Cambrex BioWhittaker Cell Biology Products, USA) and MVECs from passage 4 (rather than 3) were used for proliferation experiments.

EC migration by synthetic peptides:

EC migration was evaluated as described in Example 2 of the Examples section. Following trypsinization, ECs (25000) were incubated in migration chambers. Synthetic peptides were added at 5, 10, 20 and 50 ng/ml in the feeder tray for chemoattractant migration assay. For activation migration assays, synthetic peptides at 0.1, 1 a 10 ng/ml were incubated with the cells in migration chamber. Time of incubation in both cases was 5 and 15 hours.

MVECs migration was evaluated as described above. MVECs from passage 3 were incubated in EBM-MV supplements free media. Synthetic peptides were added at 10 ng/ml to the feeder tray for chemoattractant migration assay as well as for activation of migration assays. Results were determined in a Fluorescent ELISA reader at 480/520nm (Fluostar BMG Lab Teck).

Sprouting of aortic rings by synthetic peptides:

Human mammary or radial artery was prepared in a 96-well plate as described in Example 2 of the Examples section. Different peptide concentrations (i.e., 1, 10, 100 and 1000 ng/ml) were added to each well with the aortic ring. Plates were incubated at 37 °C with 5 % CO_2 for 7 days. Arterial rings were removed and cell proliferation was estimated by the XTT assay (Biological Industries, Kibbutz Beit Haemek, Israel) according to manufacturer instructions.

Tube Formation assay:

ECs from passage 3 or MVECs from passage 4 were harvested with trypsin and incubated in media free supplements for 24 hours. 24-well plates were pre-coated with 250 µl Cultrex Basement Membrane Extract with reduced growth factors (R&D systems, Minneapolis, USA). 500 µl medium containing 10^6 cells was transferred to the coated wells. Synthetic peptides, VEGF, YR, QF and VL, were added to ECs, and FGF, YR, QF and VL were added to MVECs at 10 ng/ml. Plates were incubated at 37

°C with CO_2 for 24 hours. Photos were taken at 20 hours for HUVECs and at 8 hours for MVECs in a light microscope.

Real Time PCR:

MVECs from passage 3 were incubated in EBM-MV supplements free media (starving media) for 24 hours. After 24 hours starvation in serum free medium, 1 ng/ml of synthetic peptides (LT, QF, SP, TR, YR, and VL) and 10 ng/ml of VEGF were added to MVEC plates. Following 1.5 and 6 hours incubation, total RNA was extracted using TRIsol reagent (Invitrogen life technologies). 0.8 µg of the extracted RNA was used as a template for reverse transcription reaction (Invitrogen life technologies), using random primers (SuperScript III First-Strand Synthesis System for RT-PCR) following manufacturer instruction protocol. Resulting cDNAs were PCR-amplified in a Real Time PCR using ABI prism 7000 Sequence Detection System (Applied Biosystems, CA, USA) in a final volume of 20 µl, using oligonucleotide primers (designed using Primer Express software, Applied Biosystems, CA, USA) complementary to a translated region of VEGF-A (Accession No:NM_003376), VEGF-C (Accession No:NM_005429), KDR (Accession No:NM_002253), FLT-1 (Accession No:NM_002019), HIF-a (Accession No:HSU22431), and GAPDH (Accession No:NM_002046) as described in Table 4.

A reaction mixture of 20 µl consisting of distilled water, oligonucleotide primers (500 nM), cDNA (3 µl), and SYBR green PCR master kit (Applied Biosystems, CA, USA) was subjected to an amplification program of 15 seconds at 95 ^{0}C, and 60 seconds at 60 ^{0}C for 40 cycles. Results were analyzed using the Sequence Detector Software Version 1 (Applied Biosystems, CA, USA).

Table 4. Oligonucleotide primers for amplification of selected genes cDNA

Gene name	Sense primer	SEQ ID NO:	Anti-sense primer	SEQ ID NO:
VEGF-A	CTACCTCCACCATGCCAAGTG	15	TGCGCTGATAGACATCCATGA	16
VEGF-C	TTCCTGCCGATGCATGTCTA	17	TGTTCGCTGCCTGACACTGT	18
KDR	TCAGGCAGCTCACAGTCCTAGAG	19	ACTTGTCGTCTGATTCTCCAGGTT	20
FLT-1	TCAGCGCATGGCAATAATAGA	21	ACCAAGGTGCTAGCCATCTTATTC	22
HIF-a	AGTGTACCCTAACTAGCCGAGGAA	23	GCCTGTGCAGTGCAATACCTT	24
GAPDH	GTCGGAGTCAACGGATTTGG	25	GGCAACAATATCCACTTTACCAGAGT	26

Statistical and graphical methods:

See Example 1 of the Examples Section.

RESULTS

Synthetic peptides chosen:

Six synthetic peptides were synthesized in order to evaluate their ability to induce *in vitro* and *in vivo* angiogenesis. The peptides designated LT, QF, SP, TR, VL, and YR are described in Table 3, above.

Specific binding of the above-described synthetic peptides to ECs was tested. The peptides were FITC-labeled and the binding was analyzed by FACS (FACScan Beckton Dickinson, CA, USA). The results obtained indicated that the synthetic peptides bind specifically to ECs, and not to lymphocytes (Figure 7). Increasing concentrations of peptides (Figure 6, red lines) resulted in increased binding to ECs (94-96 % binding) as compared to a lower concentration (Figure 6, green lines).

Induced proliferation by synthetic peptides:

The effect of synthetic peptides on proliferation of HUVEC was tested. ECs were seeded on 24 well plates in serum free media (SFM) for 24 hours, and then synthetic peptides were added to cells in various concentrations for additional 24 hours. Figure 8a demonstrates a significant increase in thymidine uptake in ECs incubated with the peptides (LT, SP, TR and VL) in a dose dependent manner. LT, TR and SP induced the highest proliferation response (1.7, 1.8 and 1.6 fold, respectively) at 10 ng/ml, while VL induced the highest proliferation response at 1 ng/ml 1.8 fold increase.).

A significant increase in thymidine uptake was also demonstrated for MVECs incubated with LT, SP or YR (Figure 8b). The increase was in a dose dependent manner with the highest proliferation response in a concentration of 1 ng/ml for all 3 peptides. At this concentration, LT YR and SP increased MVECs proliferation response by 1.8, 1.7 and 1.4 fold, respectively (Figure 8b).

Cells migration by synthetic peptides:

Migration of cells can be considered as a function, and thus an indication, of activation and attraction by peptides. The effect of the synthetic peptides added at 5, 10, 20, and 50 ng/ml to ECs incubated in the feeder tray for 5 hours on induced migration by chemical attraction was tested. A dose dependent induced migration was observed for LT (Figure 9a) and SP (Figure 9b). The effect appears to reach a plateau at high concentrations, which would be predicted based on pharmacokinetics. The exact pharmacologic profile of this attraction requires further study. A smaller effect

of induced migration was noted for VL and TR when used as chemoattractants (Figure 9c).

Experiment performed with the six selected peptides demonstrates their effectiveness in activating migration. Figure 10 illustrates that each of these peptides, within a 5 hours span, induces statistically significantly more ECs migration than control ECs without the peptide present. By 15 hours, however, the migration of cells has been reduced, so that no statistical difference is seen between any peptide-treated cells and control epithelial cells (Figure 10).

MVECs were also shown to migrate due to synthetic peptides induction. The experiment performed with the test peptides demonstrated their effectiveness as chemoattractants to induce migration of MVECs in a dose dependent manner. Figure 11a illustrates that each of the test peptides, within a 5 hours span, induced more cell migration than control endothelial cells without the peptide present (results are in net values from which the control values were subtracted). At 10 ng/ml, LT, QF, YR SP, TR and VL induced a 3.5, 2.4, 2.4, 2, 2, and 2 fold increase in MVECs migration respectively.

MVECs were also directly activated by the synthetic peptides. TR, SP, QF, and YR were all shown to induce MVECs migration (by 2.6, 2.1, 2.3 and 1.8 folds, respectively), at 1 ng/ml (Figure 11b).

Aortic rings sprouting by synthetic peptides:

As described before for the phage bound peptides, aortic rings induced sprouting was evaluated by addition to the cultured aortic rings purified synthetic peptides at different concentrations. Four peptides (QF, YR, LT, and VL) were compared for their ability to induce cell proliferation in aortic rings. Clear differences between the peptides (after correction for control optical density), with decided effects of concentration were observed (Figure 12).

Tube Formation:

ECs and MVECs were incubated on matrigel in the presence of peptides and array formation was analyzed. Peptides (YR, QF and VL) added to MVECs at concentration of 10 ng/ml, resulted in a significant increase in tube formation (Figures 13c-e) as compared to untreated cells (Figure 13a). This increase was similar to the effect of VEGF on these cells (Figure 13b). The same effect of increased tube formation was induced by these peptides when added to ECs (Figure

13h-j) as compared to untreated cells (Figure 13f). This increase was similar to the effect of FGF on ECs (Figure 13g).

As described for the phage presenting peptides, the synthetic peptides produced, could induce *in vitro* angiogenic effects in ECs. In all angiogenic effects tested, at least one (while in most cases more) of the synthetic peptides tested showed significant effect over control. These results indicate that these peptides may be also capable of *in vivo* angiogenesis.

Peptides effect on gene expression in MVECs:

The effect of the synthetic peptides on the expression of selected genes (VEGF-A, VEGF-C, KDR, FLT-1 and HIF-1a) related to the VEGF pathway (a major pathway that participated in angiogenic process) in MVECs was tested. Synthetic peptides were added at 1 ng/ml for 1.5 and 6 hours. After incubation with peptide, RNA was extracted from MVECs and Real Time PCR was performed. Gene expression was calculated as peptide / control (cells without peptide treatment) ratio.

The different peptides had varying effects on the expression of the genes tested as demonstrated in Figure 14 and summarized in Table 5.

Table 5. The effect of the different peptides on gene expression

Gene	Synthetic peptide	Expression after 1.5 hours	Synthetic peptide	Expression after 6 hours
VEGF-A (Figure 14a)	QF, TR, YR, VL, LT, SP	+*	VL QF, TR, YR	+ -**
VEGF-C (Figure 14b)	QF, TR, VL, LT, SP	+	QF, TR, VL, LT, SP	+
FLT-1 (Figure 14c)	QF, TR, YR, VL, LT, SP	+	QF, YR	-
KDR (Figure 14d)	QF, SP, YR LT	+ -	TR, VL YR	+ -
HIF (Figure 14e)	QF, TR, VL LT, YR	+ -	TR, VL LT, QF	+ -

* "+" Increased gene expression

** "-" Reduced gene expression

Among the 6 peptides tested, all were shown to induce the expression of some of the genes tested. These results may lead to the molecular mechanism by which these peptides induce angiogenesis.

EXAMPLE 4

The effect of hypoxia on in vitro induced angiogenesis by synthetic peptides.

The ability of selected peptides to induce angiogenesis was evaluated by induction of cells binding, proliferation, migration and tube formation assays under hypoxic condition.

MATERIALS AND METHODS

Peptides synthesis and Fluorescein labeling:

See example 3 of the Examples section.

ECs and hypoxia conditions:

ECs were isolated, cultured, and subjected to hypoxia conditions as described in Example 1 of the Examples section.

FACS analysis of peptide binding to ECs with and without hypoxia treatment:

ECs were exposed to hypoxia conditions and then prepared for FACS analysis as described in Example 3 of the Examples section. Cells were stained with 6μg of SP or LT labeled peptide. Samples were analyzed by Fluorescence Activated Cell Sorter (FACScan Beckton Dickinson, CA, USA).

Synthetic peptides induced proliferation after and under hypoxia treatment:

Cells proliferation assays were performed as described in Example 3 of the Examples section but for proliferation assays cells were divided to 3 groups. One, control group, the second group was of cells after exposure to hypoxia conditions, and the third group, were cells proliferating under hypoxia conditions. ECs were incubated with QF, LT and SP and MVECs were incubated with LT, SP, and YR

Synthetic peptides induced migration after and under hypoxia treatment:

EC migration assays were performed as described in Example 3 of the Examples section but for proliferation assays cells were divided to 3 groups. One, control group, the second group was of cells after exposure to hypoxia conditions, and the third group, were cells proliferating under hypoxia conditions.

ECs were incubated with EBM-2 and MVECs were incubated with EBM-MV media.

Tube formation assay after and under hypoxia treatment:

Tube formation assay was performed as described in Example 3 of the Examples section but cells were divided to 3 groups. One, control group, the second

group was of cells after exposure to hypoxia conditions, and the third group, were cells on matrigel basement that form tubes under hypoxia treatment.

Statistical and graphical methods:

See Example 1 of the Examples Section.

RESULTS

Synthetic peptides binding to ECs after hypoxia treatment:

Peptide binding analysis to ECs and ECs after hypoxia conditions, showed that only LT and SP exhibited increased binding to ECs exposed to hypoxia. The other peptides had similar intensity of binding to ECs under both conditions (Figures 15 and 16).

Synthetic peptides induced proliferation after and under hypoxia treatment:

The effect of synthetic peptides (QF, LT and SP) on proliferation of ECs was tested after and under hypoxia treatment as compared to control. ECs were seeded on 24 well plates in serum free media for 24 hours, and then synthetic peptides were added to cells in various concentrations for additional 24 hours.

Figures 17a-b demonstrate a significant increase in thymidine uptake in ECs after and under hypoxia incubated with the peptides LT and SP, in a dose dependent manner. LT (10 ng/ml) increased 3.5 fold cell proliferation after hypoxia and 1 ng/ml increased cell proliferation under hypoxia in 1.7 fold (Figure 17a). SP (10 ng/ml) increased cell proliferation after hypoxia in 2 fold (Figure 17b). QF did not show increase in proliferation of endothelial cells after or under hypoxia treatment (Figure 17c).

The effect of these synthetic peptides (QF, LT and SP) on the proliferation of MVECs under the same conditions was also tested. Figures 17d-e demonstrate a significant increase in thymidine uptake in MVECs after and under hypoxia, when incubated with the peptides LT and SP, in a dose dependent manner. LT increased 1.7 fold MVECs proliferation after hypoxia at 1 ng/ml and at 10 ng/ml under hypoxia conditions (Figure 17d). SP increased MVECs proliferation after hypoxia in 1.5 fold at 10 ng/ml and 1.9 fold under hypoxia conditions at 1 ng/ml (Figure 17e). QF did not show increase proliferation of MVECs under hypoxic conditions compared to normoxic conditions (Figure 17f).

Tube formation assay after and under hypoxia treatment:

The effect of synthetic peptides on tube formation of ECs and MVECs was tested by their incubation on matrigel in the presence of LT, SP and QF peptides.

Addition of 10 ng/ml peptides QF under normoxic conditions, similar to the effect of VEGF or bFGF resulted in a significant increase in tube formation in comparison to untreated ECs (Figure 13d and i). SP and LT under normoxic conditions did not induce tube formation (Data not shown). Peptide SP however, was effective only in tube formation under hypoxia conditions (Figure 18a-e).

EXAMPLE 5

Peptide- induced in vivo angiogenesis.

The angiogenic synthetic peptides were used to induce *in vivo* angiogenesis in a mouse ear model and a rat ischemic hind-limb model.

MATERIALS AND METHODS:

In vivo angiogenesis in a mouse-ear model:

Ear angiogenesis studies were a modification of an approach described previously (Pettersson, 2000). Synthetic peptides in a concentration of 1, 10 and 20 μg/15 μl per mouse were injected sub-cutaneously into the ears of nude mice and Balb/C mice. Contralateral ears were injected only with PBS. Digital photographs were obtained 2, 4, 6, and 20 days after injection. Two days after peptide inoculation, angiogenic effect of peptides could be observed.

Histological sections:

Histological sections of the mouse-ears injected with the angiogenic peptides were performed by fixing tissues in 4 % buffered formalin. Sections were embedded in paraffin blocks sectioned in 4 μm thick layers and stained with hematoxolin-eosin.

Rat ischemic hind-limb model and laser-Doppler imager analysis:

A rat ischemic hind limb model was used for evaluation of the *in vivo* potential of angiogenesis induced by the selected synthetic peptides. Ischemia was created in the rat left hind limb by ligation the femoral artery. The right hind limb served as a control. A day after the operation each of the peptides was injected into two sites close to the ligation and one site distal to the ligation. Each rat was treated with each of the peptides in a total amount of 600μg.

The blood flow was measured using a Laser Doppler Blood Flow analyzer (moorLDI, Moor Instrument, Wilmington, Delaware) at 2, 6, 9 and 13 days after peptides injections. The average perfusion of each limb was computed and blood flow was expressed as the ischemic (left) / control (right) blood flow ratio.

Statistical and graphical methods:

See Example 1 of the Examples Section.

RESULTS:

In vivo angiogenesis in a mouse-ear model:

Injection of the synthetic peptides into the ears of nude mice and Balb/C mice resulted in increased number of blood vessels in the ears of mice injected with 10 μg of LT, YR, QF and SP (Figure 19 b-e). Histological examination of stained sections of the ears revealed an increase in the number of blood vessels and the appearance of neo-vascularizations in peptide injected ears (Figure 20, Table 6).

Table 6. Blood vessels induced by peptide injection

Peptide	Concentration	Days after inoculation	Number of blood vessels
FGF	3 ng/ear	5	11
control		5	7
VEGF	10 ng/ear	5	17
control		5	10
LT	10 μg/ear	5	18
control		5	13
SP	10 μg/ear	5	15
control		5	9
YR	10 μg/ear	5	11
control		5	12
TR	10 μg/ear	5	15
control		5	9
QF	10 μg/ear	5	21
control		5	15
VL	10 μg/ear	5	14
control		5	12

Laser-Doppler analysis in a rat ischemic hind-limb model:

The blood flow of ischemic hind limb was measured after 600 μg peptide injection. The blood flow was measured using a Laser Doppler Blood Flow analyzer (MoorLDI, Moor Instrument, Wilmington, Delaware) at 4 time points (at days 2, 6, 9 and 13). The percent of median flux of the operated leg / control leg of rats treated with peptides was calculated for each peptide injected. Treatment of rats with the peptides QF and YR showed 112.5 and 108.2 percent increase of median flux of the ischemic leg / control leg, respectively (Figure 21).

EXAMPLE 6

A conserved sequence motif (SEQ ID NO: 13) supports the angiogenic function attributed to the peptides of the present invention.

Sequence analysis of the isolated peptides revealed a conserved amino acid sequence which is shared by 3-4 of the peptides VL; QF, TR and possibly YR (see Figure 22).

This sequence was found by the eMOTIF scan software (Biochemistry, Stanford University, http://dna.stanford.edu/emotif/emotif-scan.html) to be shared with mouse vascular endothelial growth factor B precursor (Swiss-Prot Accession: VEGB_MOUSE), which has a very similar human homologue. The following peptide sequences YR(shared); LT; and SP may belong to a different group. Interestingly these two groups of peptide were isolated under two different test conditions, while the first (VL, QF and TR) were isolated under normoxic conditions the second groups of peptides (YR, LT and SP) were selected under hypoxic conditions, suggesting that these two groups bind to different cellular determinants or with different affinities.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims. All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application

shall not be construed as an admission that such reference is available as prior art to the present invention.

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WHAT IS CLAIMED IS:

1. A peptide comprising an amino acid sequence as set forth in SEQ ID NO: 13, the peptide being at least 6 and no more than 50 amino acid residues in length.

2. The peptide of claim 1, wherein the peptide is selected from the group consisting of SEQ ID NOs: 2, 6 and 12.

3. The peptide of claim 1, wherein the amino acid sequence is selected from the group consisting of SEQ ID NOs: 2, 6 and 12.

4. The peptide of claim 1, wherein the peptide is a linear peptide or a cyclic peptide.

5. A peptide selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10 and 12.

6. The peptide of claim 5, wherein the peptide is a linear peptide or a cyclic peptide.

7. A peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10 and 12, the peptide being no more than 50 amino acid residues in length.

8. The peptide of claim 7, wherein the peptide is a linear peptide or a cyclic peptide.

9. A composition-of-matter comprising at least two peptides, each independently selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10 and 12.

10. A pharmaceutical composition comprising a therapeutically effective amount of a peptide having an amino acid sequence as set forth in SEQ ID NO: 13,

said peptide being at least 6 and no more than 50 amino acid residues in length and a pharmaceutically acceptable carrier or diluent.

11. The pharmaceutical composition of claim 10, wherein said peptide is selected from the group consisting of SEQ ID NOs: 2, 6 and 12.

12. The pharmaceutical composition of claim 10, wherein said amino acid sequence is selected from the group consisting of SEQ ID NOs: 2, 6 and 12.

13. The pharmaceutical composition of claim 10, wherein said peptide is a linear peptide or a cyclic peptide.

14. A pharmaceutical composition comprising a therapeutically effective amount of a peptide selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10 and 12 and a pharmaceutically acceptable carrier or diluent.

15. A pharmaceutical composition comprising a therapeutically effective amount of a peptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10 and 12, said peptide being no more than 50 amino acid residues in length and a pharmaceutically acceptable carrier or diluent.

16. A method of promoting angiogenesis in a tissue of a subject, the method comprising providing to the subject, a therapeutically effective amount of a peptide having an amino acid sequence as set forth in SEQ ID NO: 13, said peptide being at least 6 and no more than 50 amino acid residues in length, to thereby promote angiogenesis in the subject.

17. The method of claim 16, wherein said peptide is selected from the group consisting of SEQ ID NOs: 2, 6 and 12.

18. The method of claim 16, wherein said amino acid sequence is selected from the group consisting of SEQ ID NOs: 2, 6 and 12.

19. The method of claim 16, wherein said peptide is a linear peptide or a cyclic peptide.

20. The method of claim 16, wherein the subject suffers from arteriosclerosis, retinopathy, remodeling disorder, von Hippel-Lindau syndrome, diabetes and/or hereditary hemorrhagic telengiectasia.

21. A method of promoting angiogenesis in a tissue of a subject, the method comprising providing to the subject, a therapeutically effective amount of a peptide selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10 and 12, to thereby promote angiogenesis in the subject.

22. The method of claim 21, wherein the subject suffers from arteriosclerosis, retinopathy, remodeling disorder, von Hippel-Lindau syndrome, diabetes and/or hereditary hemorrhagic telengiectasia.

23. A method of promoting angiogenesis in a tissue of a subject, the method comprising providing to the subject, a therapeutically effective amount of a peptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10 and 12, said peptide being no more than 50 amino acid residues in length, to thereby promote angiogenesis in the subject.

24. The method of claim 23, wherein the subject suffers from arteriosclerosis, retinopathy, remodeling disorder, von Hippel-Lindau syndrome, diabetes and/or hereditary hemorrhagic telengiectasia.

25. A nucleic acid construct comprising a polynucleotide sequence encoding the peptide of claim 1.

26. The nucleic acid construct of claim 25, further comprising a promoter.

27. A nucleic acid construct comprising a polynucleotide sequence encoding the peptide of claim 5.

28. The nucleic acid construct of claim 27, further comprising a promoter.

29. A nucleic acid construct comprising a polynucleotide sequence encoding the peptide of claim 7.

30. The nucleic acid construct of claim 29, further comprising a promoter.

31. A composition for targeting a drug to endothelial cells, the composition comprising the drug fused to a peptide having an amino acid sequence as set forth in SEQ ID NO: 13, said peptide being at least 6 and no more than 50 amino acid residues in length.

32. The composition of claim 31, wherein the drug is selected from the group consisting of a toxin, a chemotherapeutic agent and a radioisotope.

33. A composition for targeting a drug to endothelial cells, the composition comprising the drug fused to a peptide selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10 and 12.

34. A composition for targeting a drug to endothelial cells, the composition comprising the drug fused to a peptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10 and 12, said peptide being no more than 50 amino acid residues in length.

35. A method of identifying putative angiogenic molecules, the method comprising:

(a) providing endothelial cells having peptides bound thereto, each of said peptides having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10 and 12, said peptide being no more than 50 amino acid residues in length; and

(b) identifying a molecule capable of displacing said peptides from said endothelial cells, to thereby identify putative angiogenic molecules.

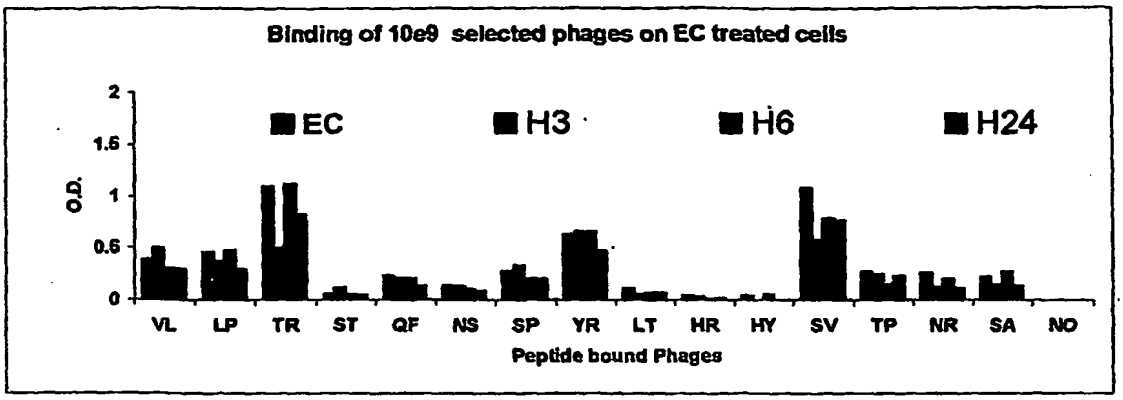
36. An antibody or an antibody fragment comprising an antigen recognition region capable of binding the peptide of claim 1, 5, 7 or 9.

ABSTRACT OF THE DISCLOSURE

A peptide comprising an amino acid sequence as set forth in SEQ ID NO: 13 is provided. The peptide being at least 6 and no more than 50 amino acid residues in length. Also provided are therapeutic applications using such peptides.

Figures 1a-b

1a.



1b.

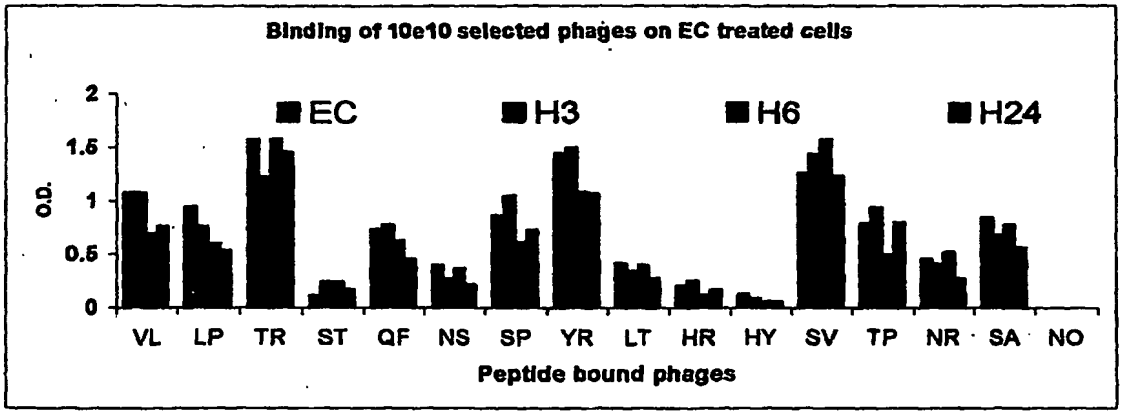
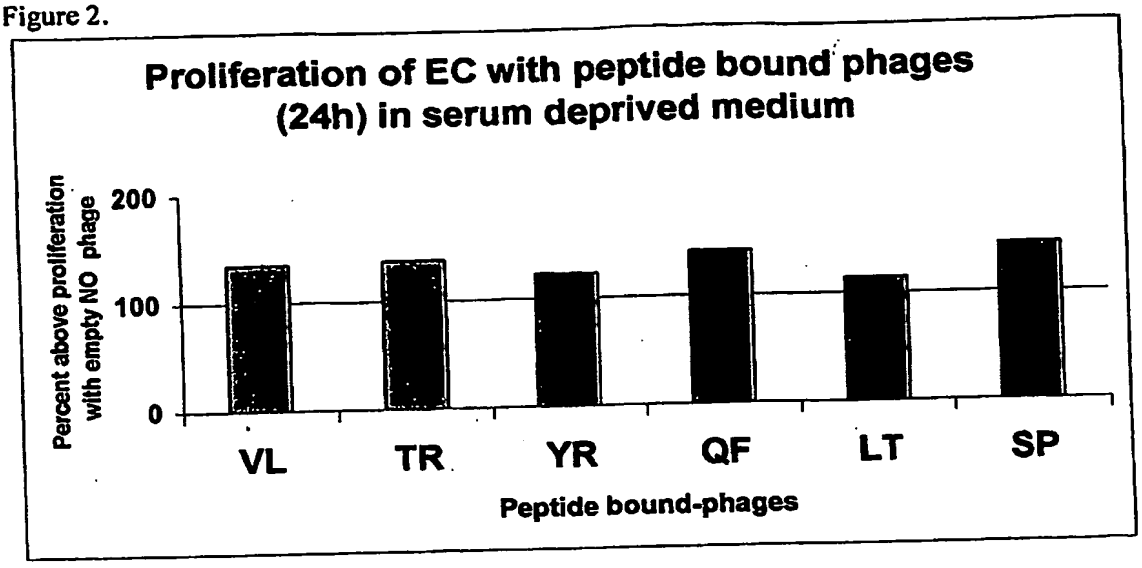
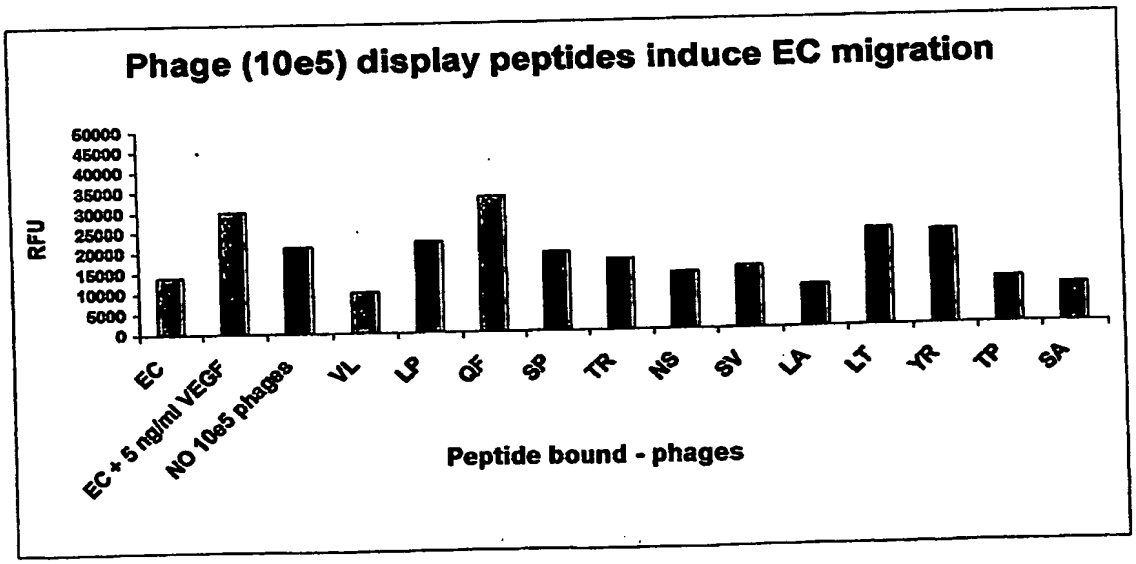


Figure 2.

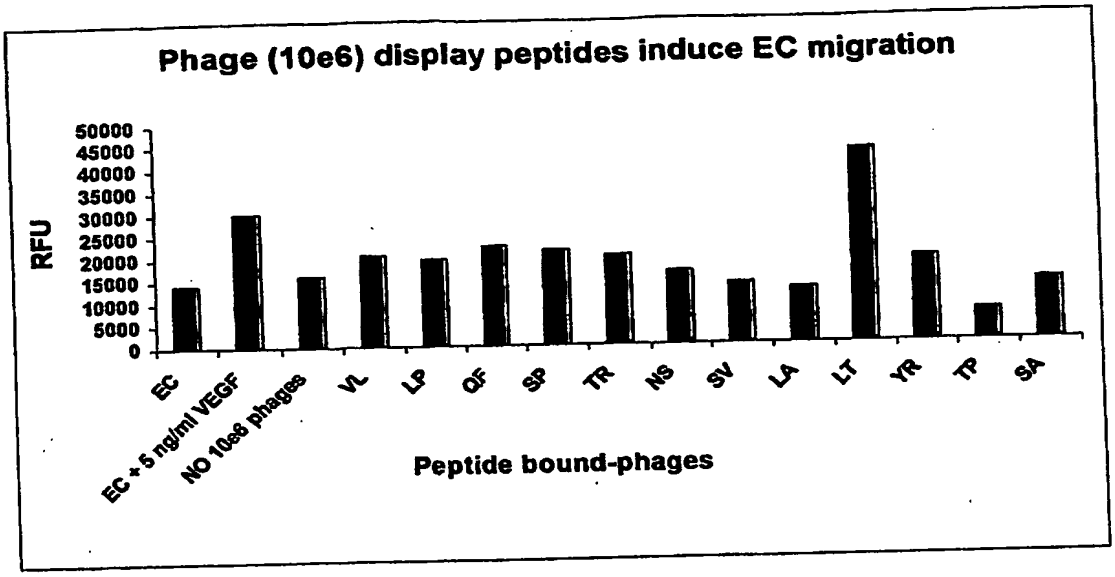


Figures 3a-b.

3A.

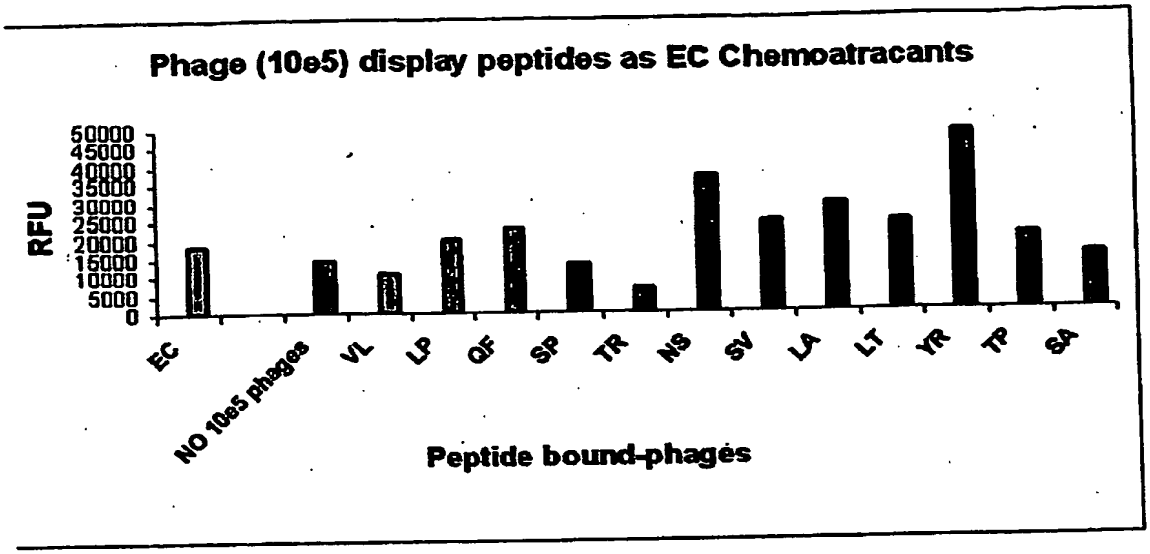


3B.


Phage (10e6) display peptides induce EC migration
RFU
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
EC
EC + 5 ng/ml VEGF
NO 10e8 phages
VL
LP
OF
SP
TR
NS
SV
LA
LT
YR
TP
SA
Peptide bound-phages

Figures 4a-b.

4A.



4B.

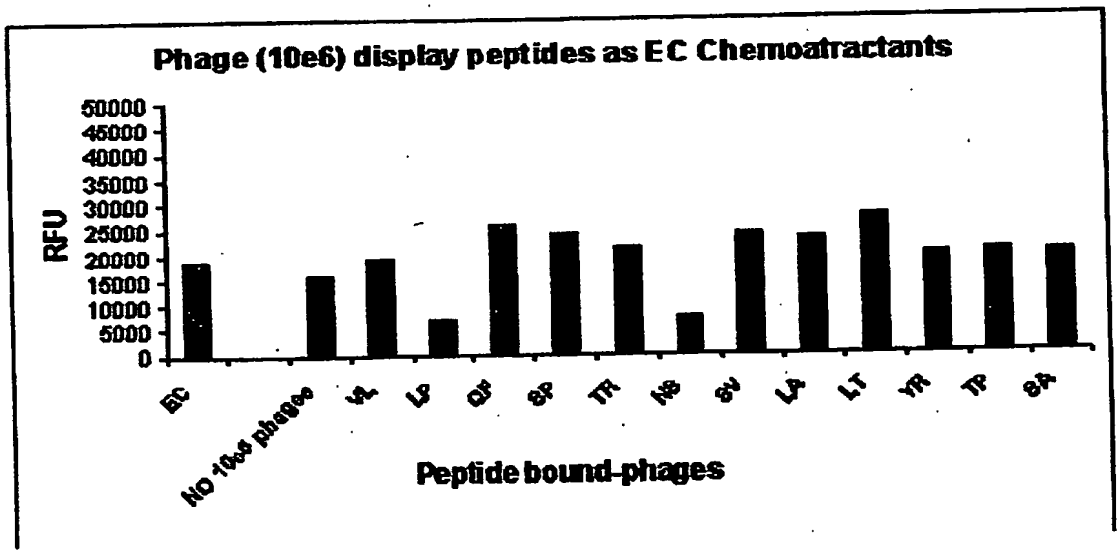


Figure 5.

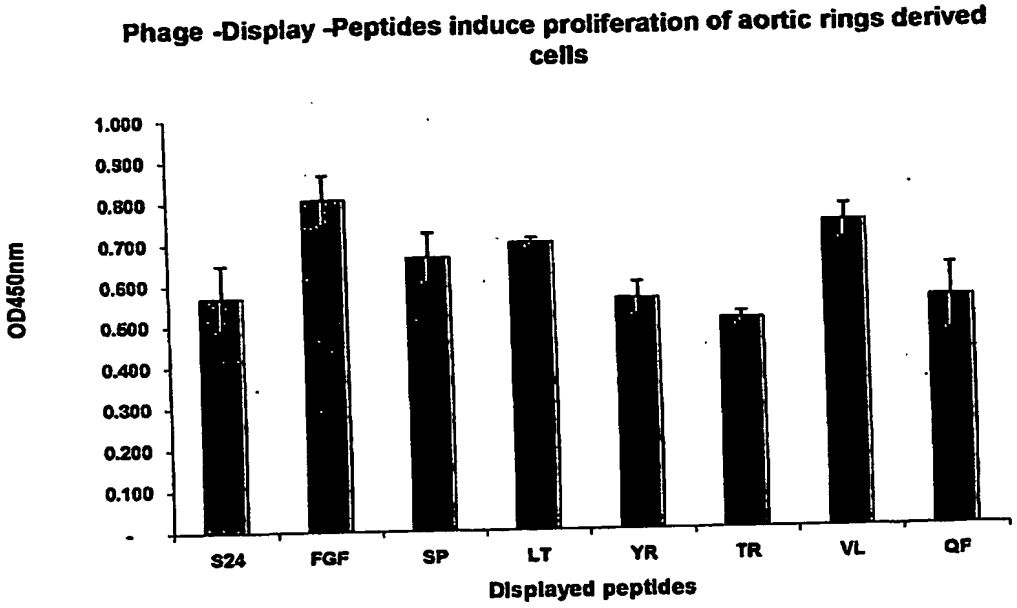

Phage -Display -Peptides induce proliferation of aortic rings derived cells
OD450nm
1.000
0.900
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
-
S24
FGF
SP
LT
YR
TR
VL
QF
Displayed peptides

Figure 6.

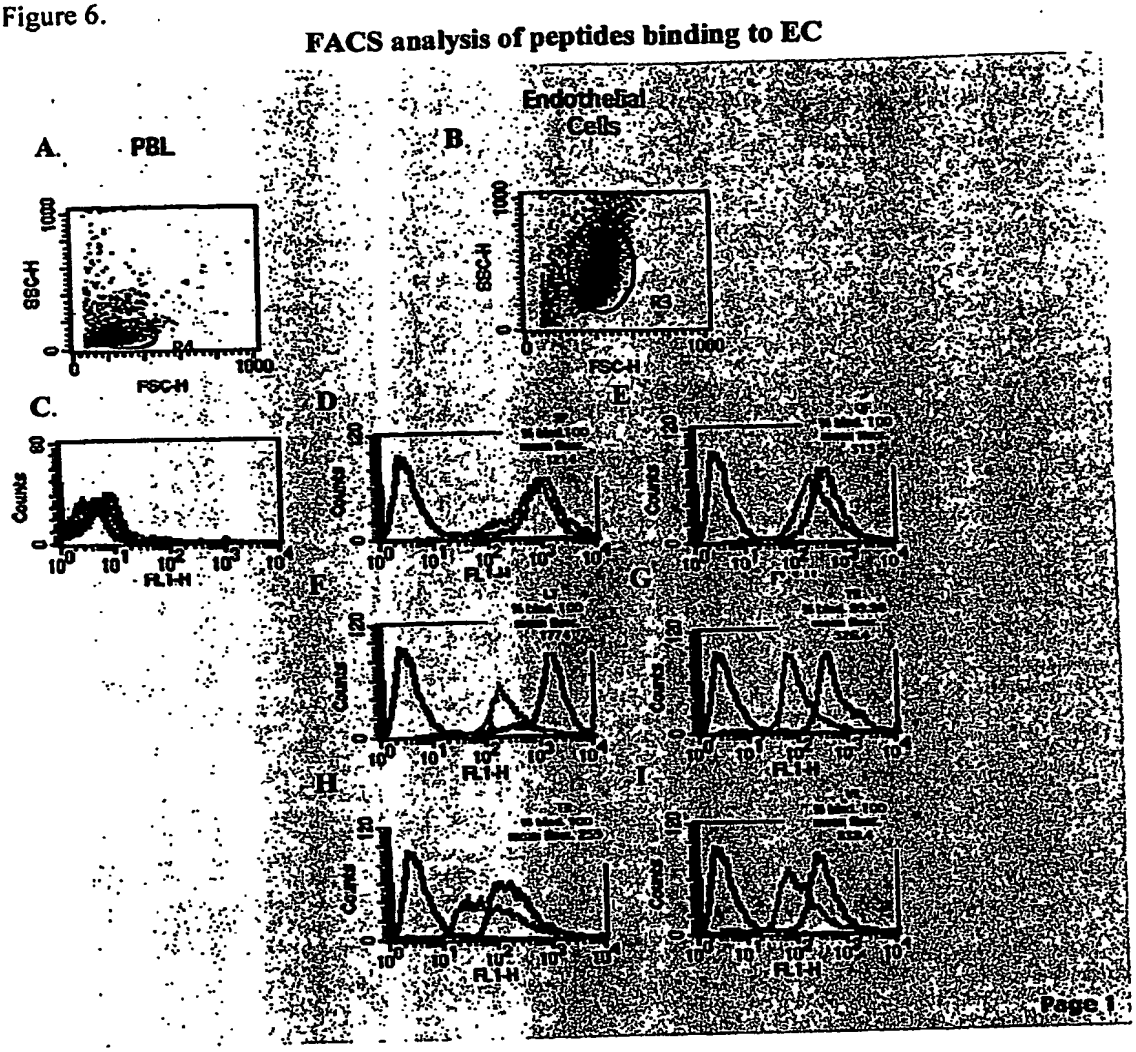
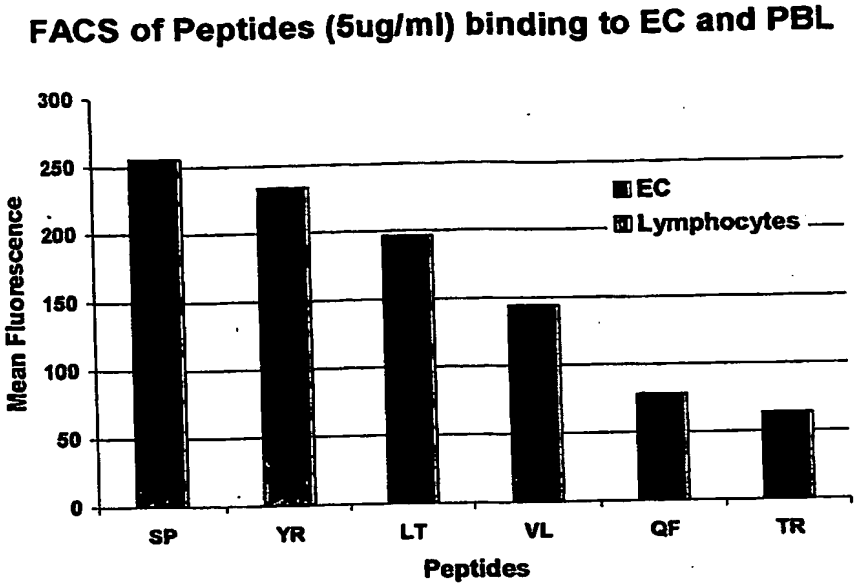
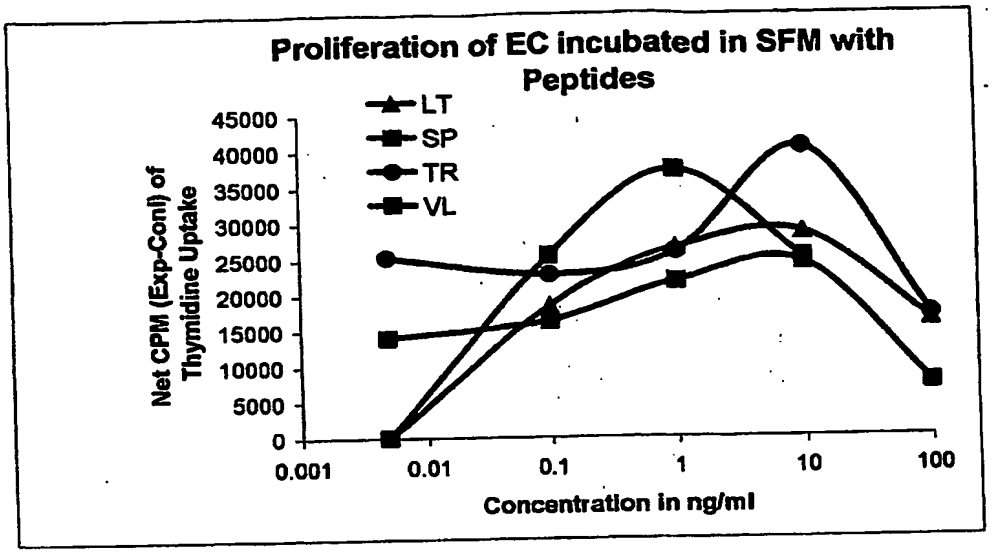

FACS analysis of peptides binding to EC
A.
PBL
B.
Endothelial Cells
SSC-H
FSC-H
R4
R3
C.
D
E
F
G
H
I
Counts
FL1-H
Page 1

Figure 7.

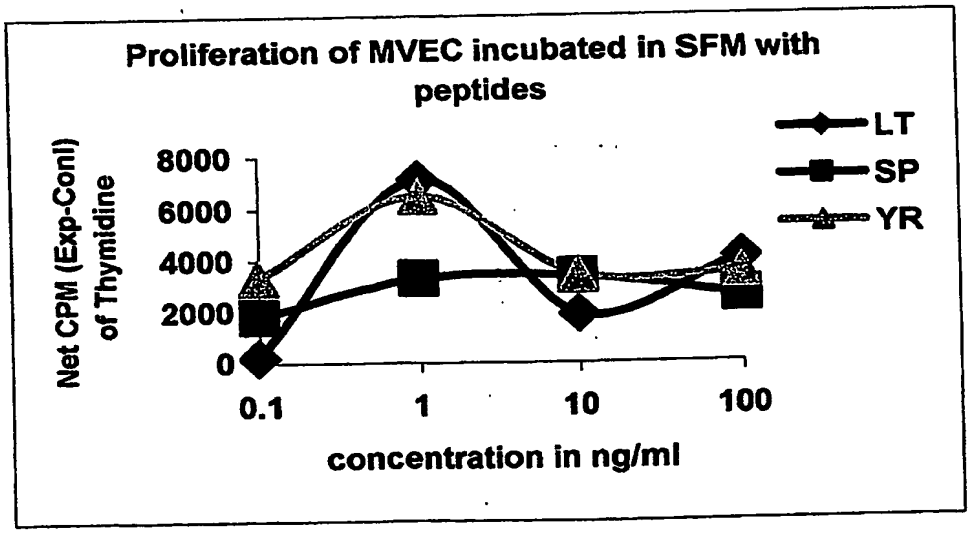

FACS of Peptides (5ug/ml) binding to EC and PBL
Mean Fluorescence
300
250
200
150
100
50
0
EC
Lymphocytes
SP
YR
LT
VL
QF
TR
Peptides

Figures 8a-b.

8A.

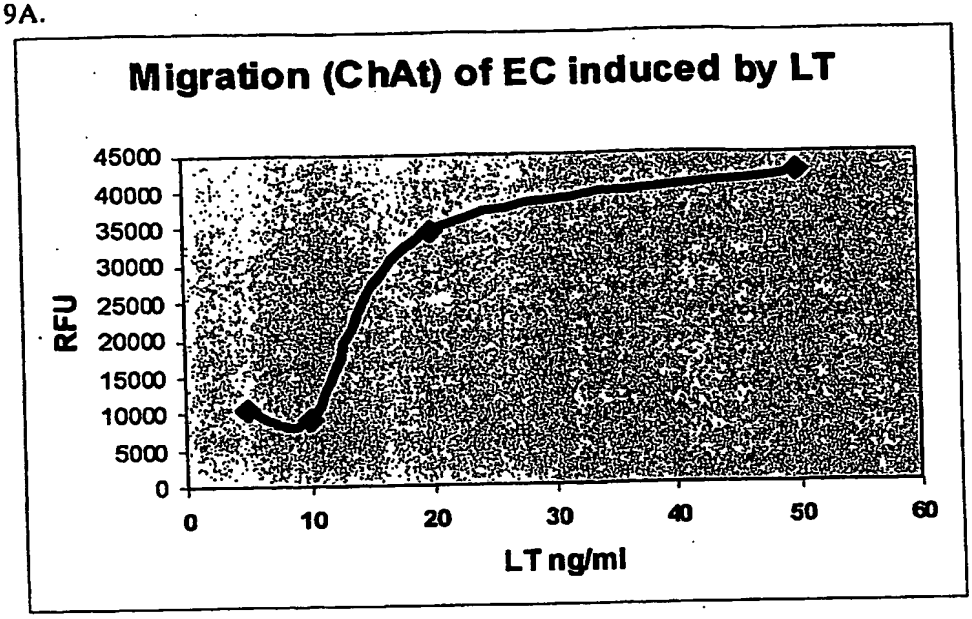


8B.

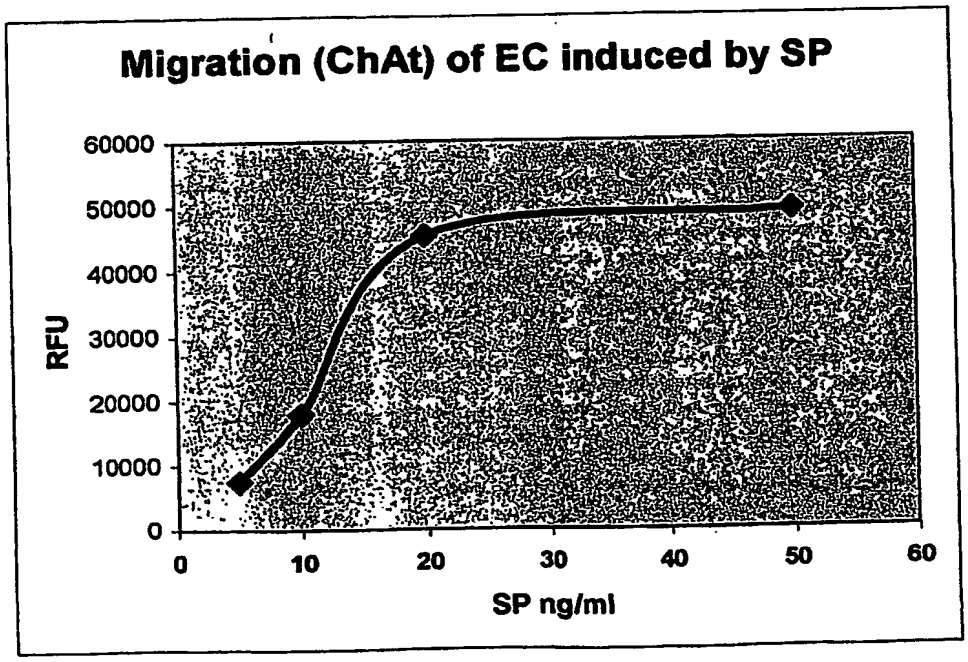


Figures 9a-c.

9A.



9B.



9C.

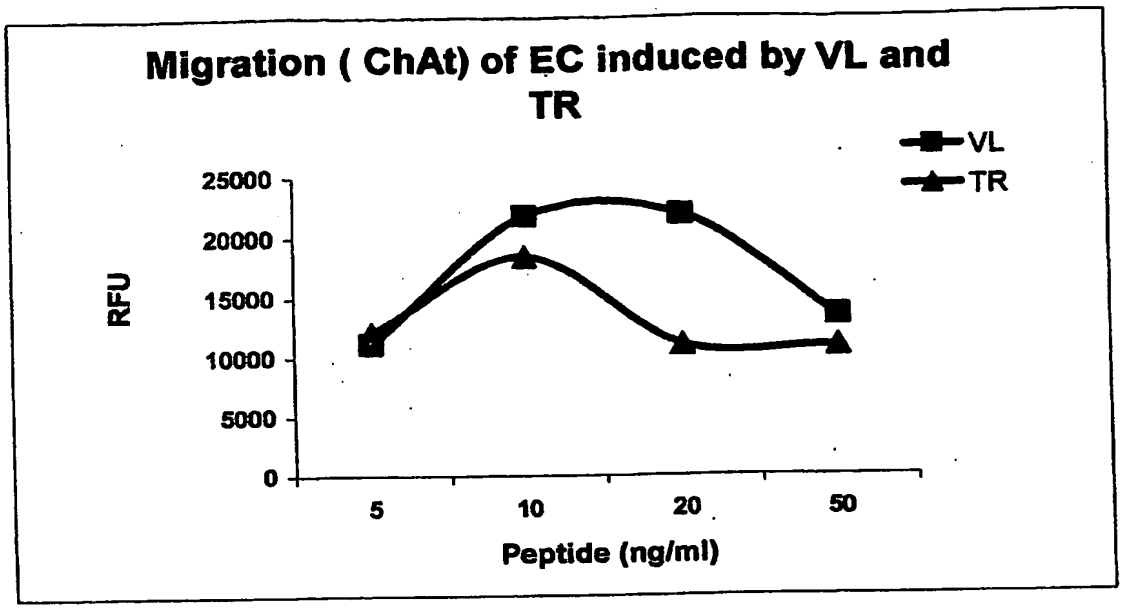
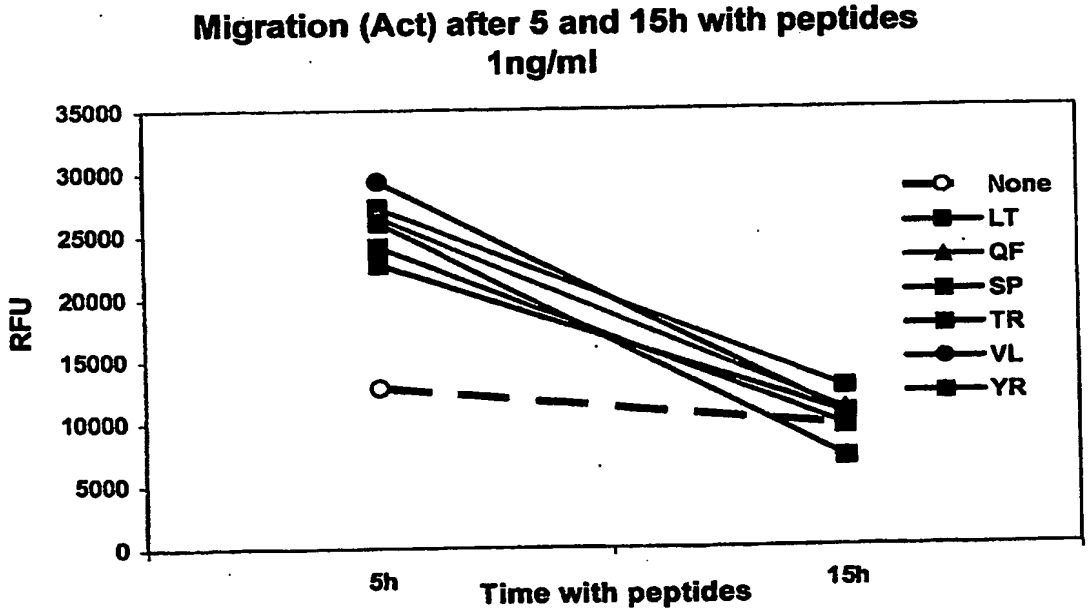
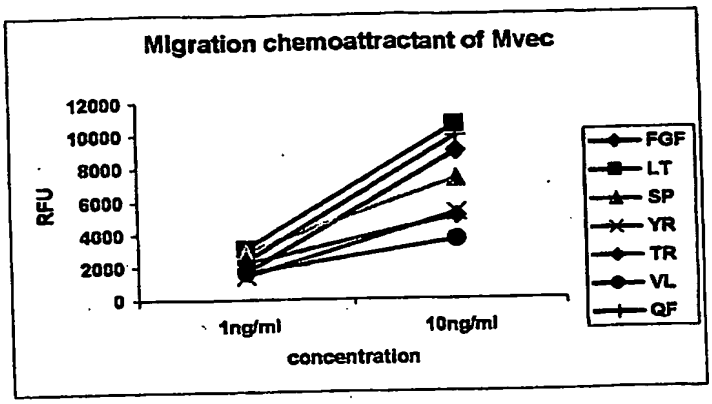

Migration (ChAt) of EC induced by VL and TR
VL
TR
RFU
25000
20000
15000
10000
5000
0
5
10
20
50
Peptide (ng/ml)

Figure 10.


Migration (Act) after 5 and 15h with peptides 1ng/ml
RFU
35000
30000
25000
20000
15000
10000
5000
0
None
LT
QF
SP
TR
VL
YR
5h
15h
Time with peptides

Figures 11a-b.

11A.



11B.

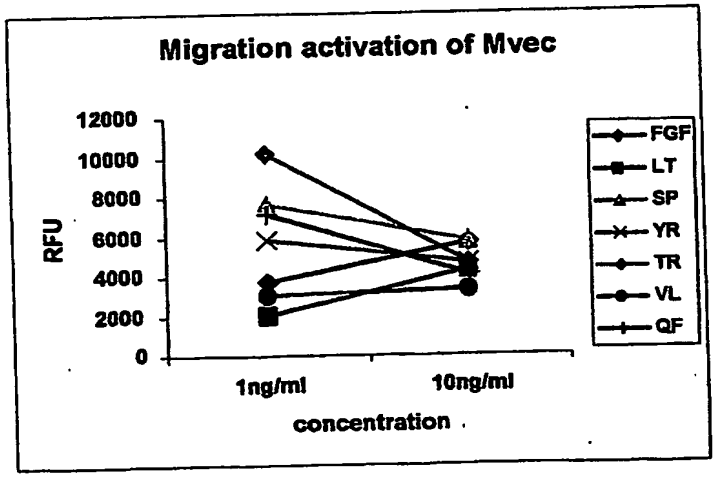
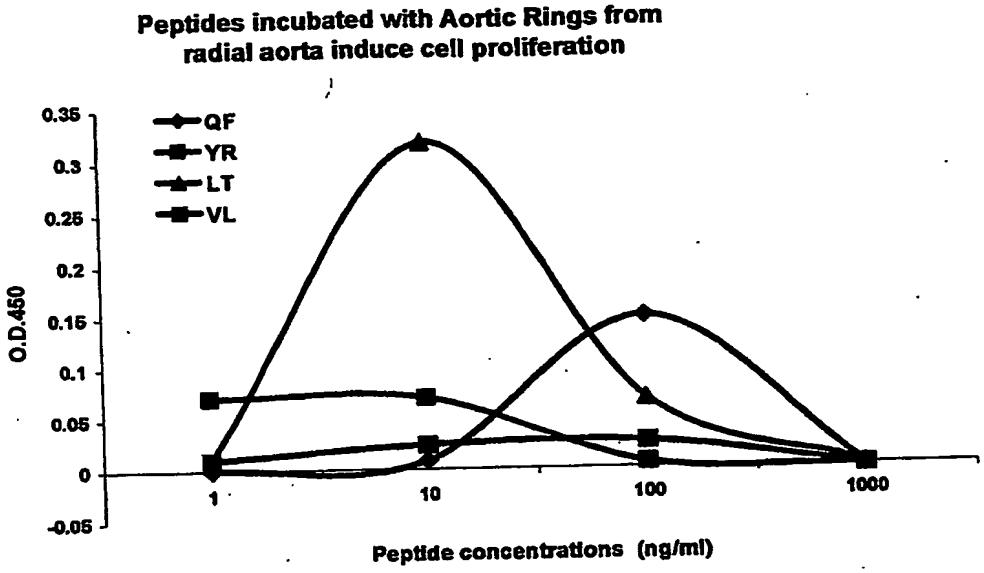


Figure 12.



Figures 14a-e

14A.

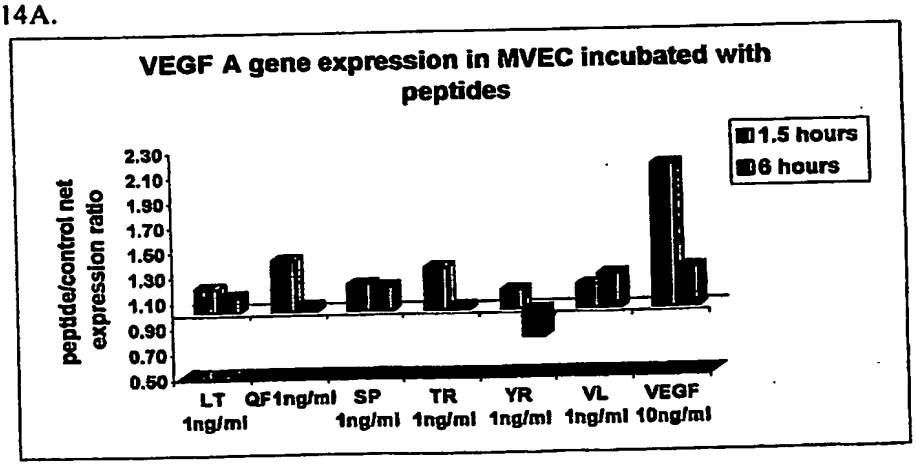
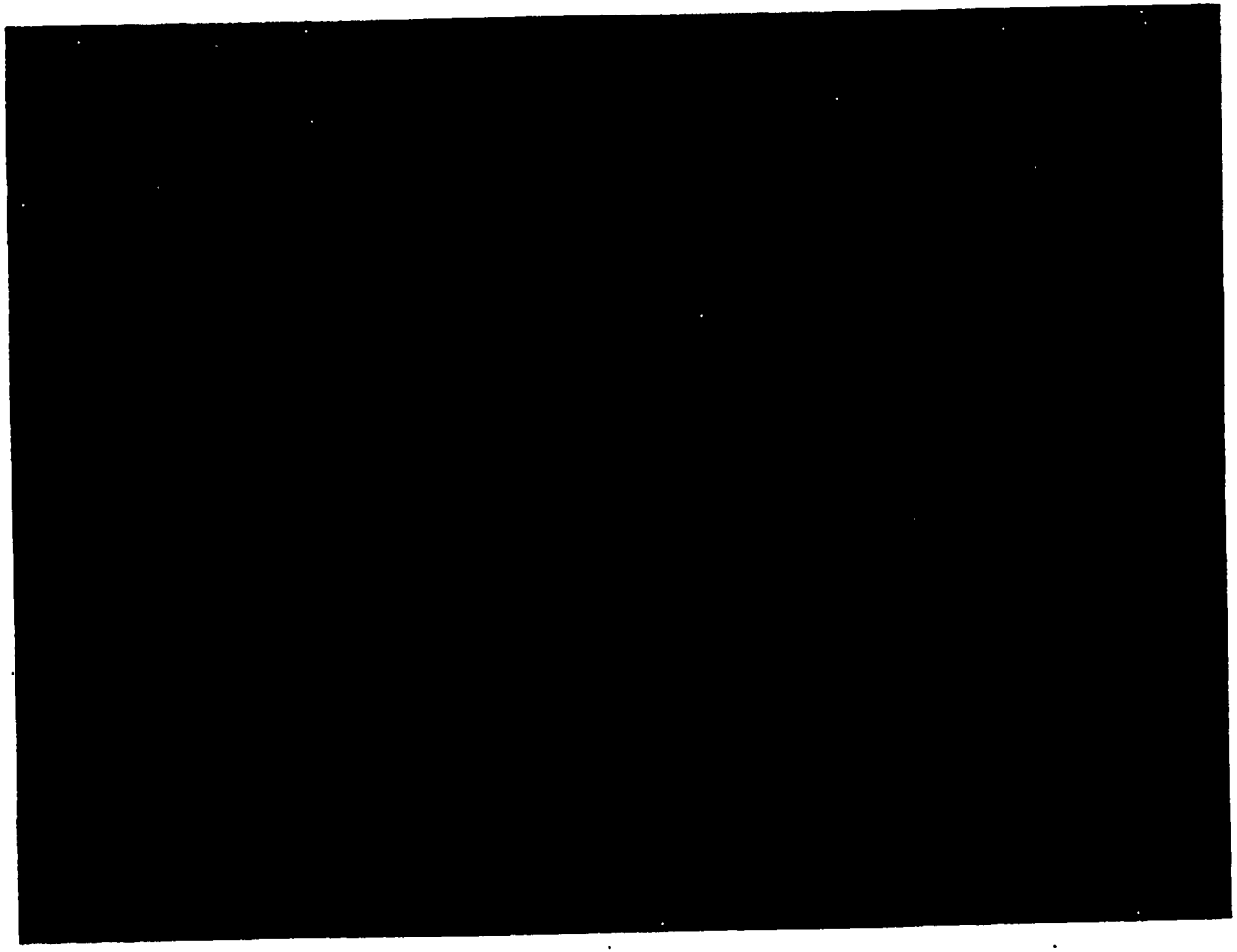
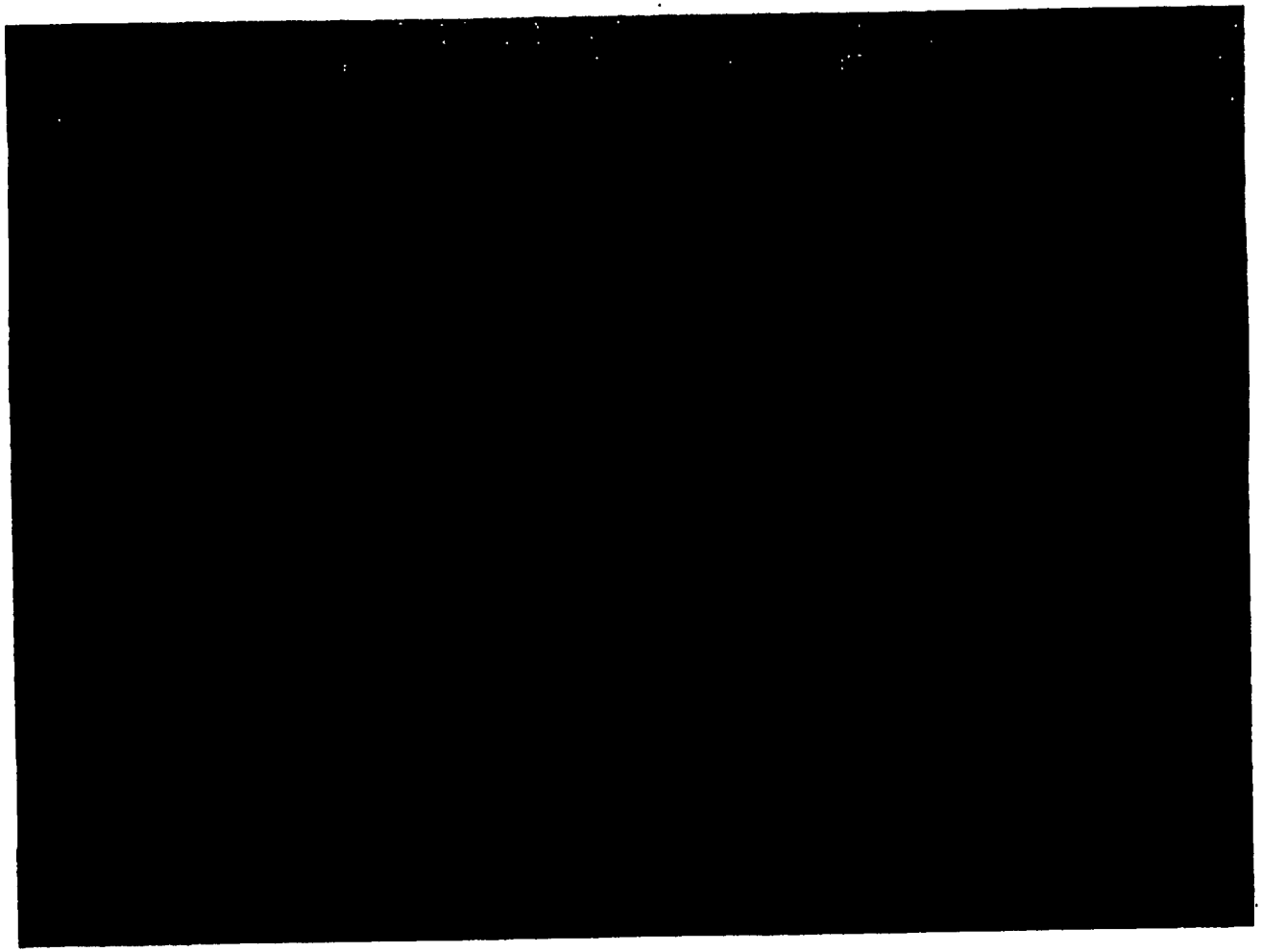


Fig 13 **A. MVEC on matrigel (x100)**



B. MVEC on matrigel with VEGF (x100)



19 13 **C. MVEC on matrigel with YR (x100)**

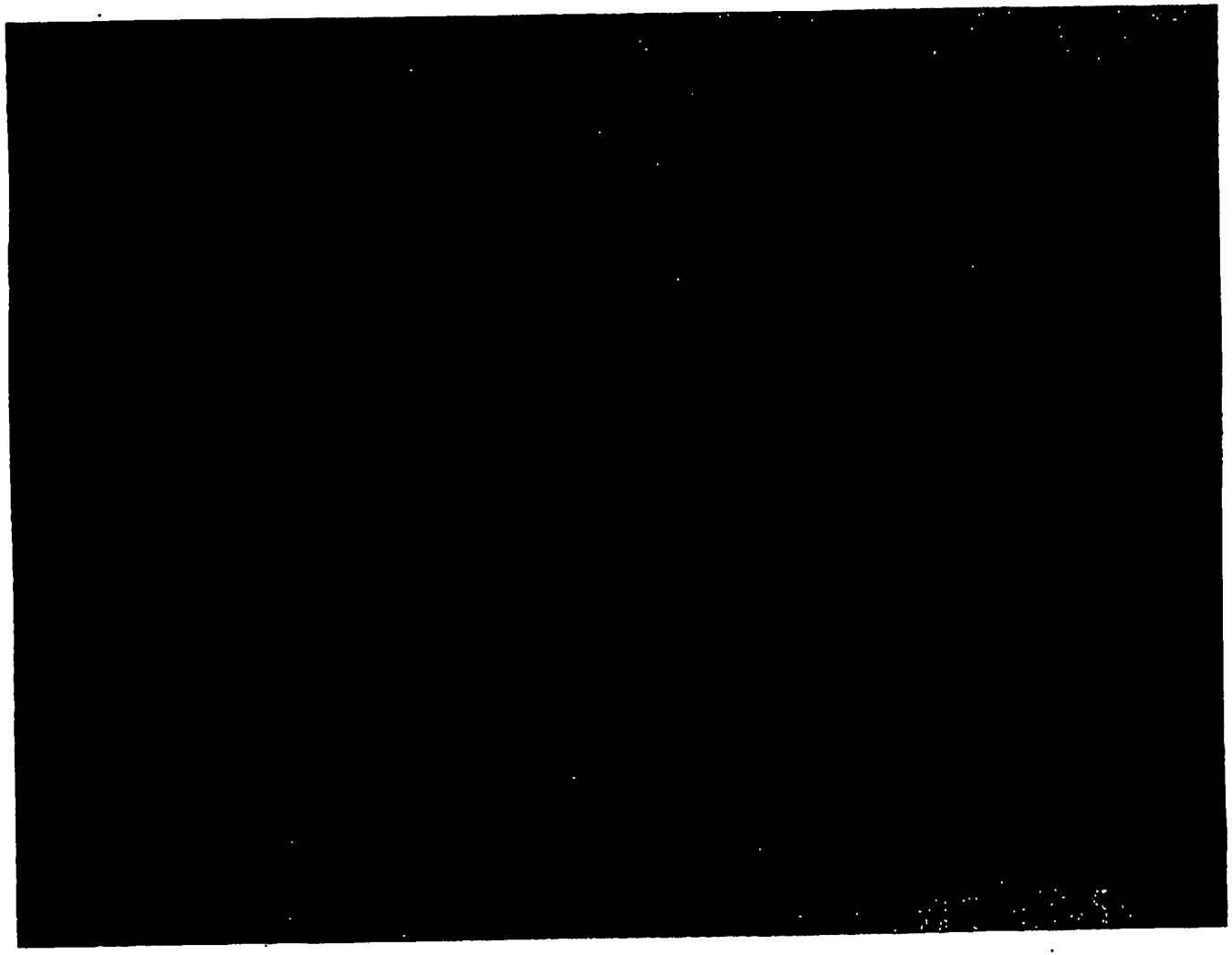


Fig 13 **D. MVEC on matrigel with QF (x100)**

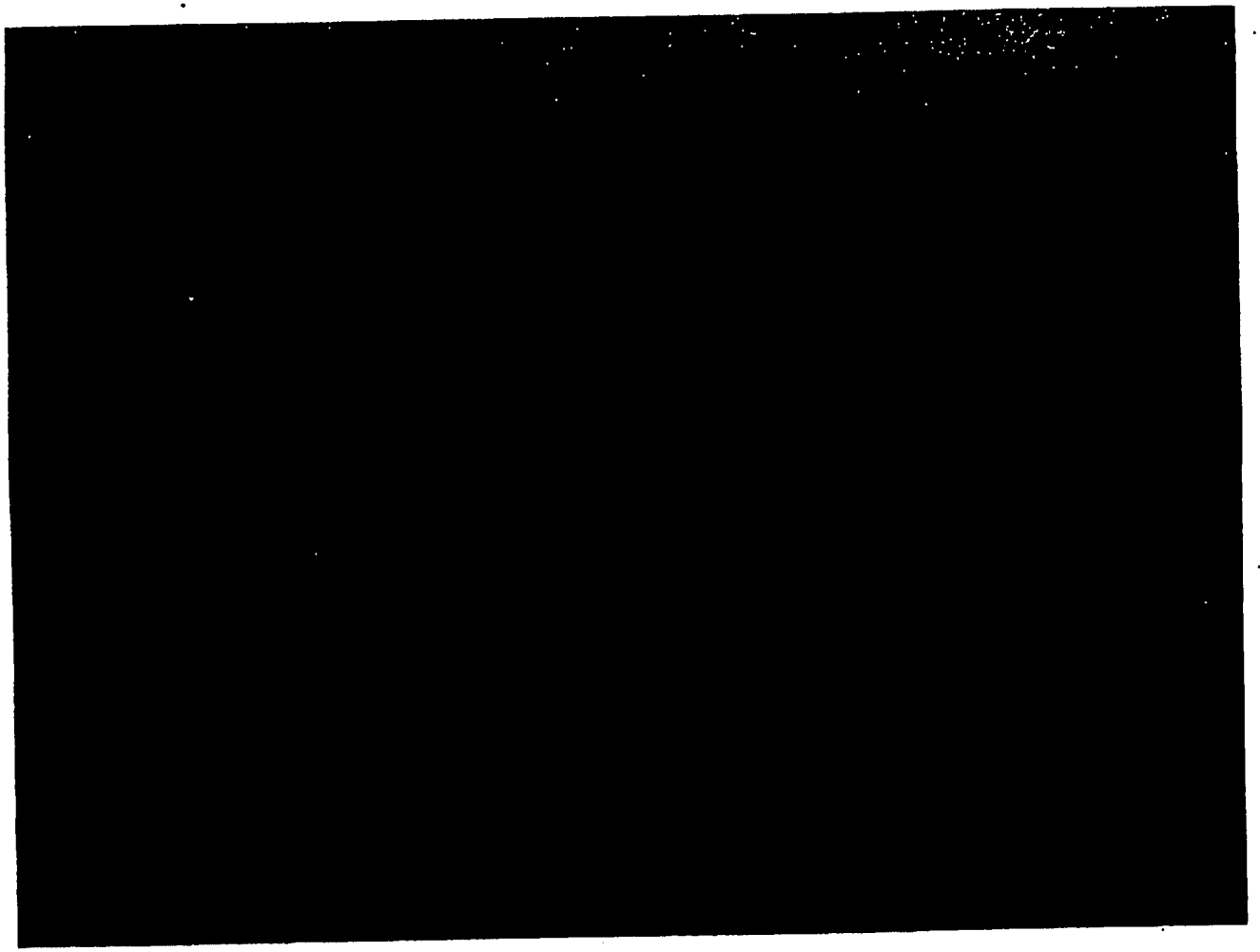


Fig 13 **E. MVEC on matrigel with VL (x100)**

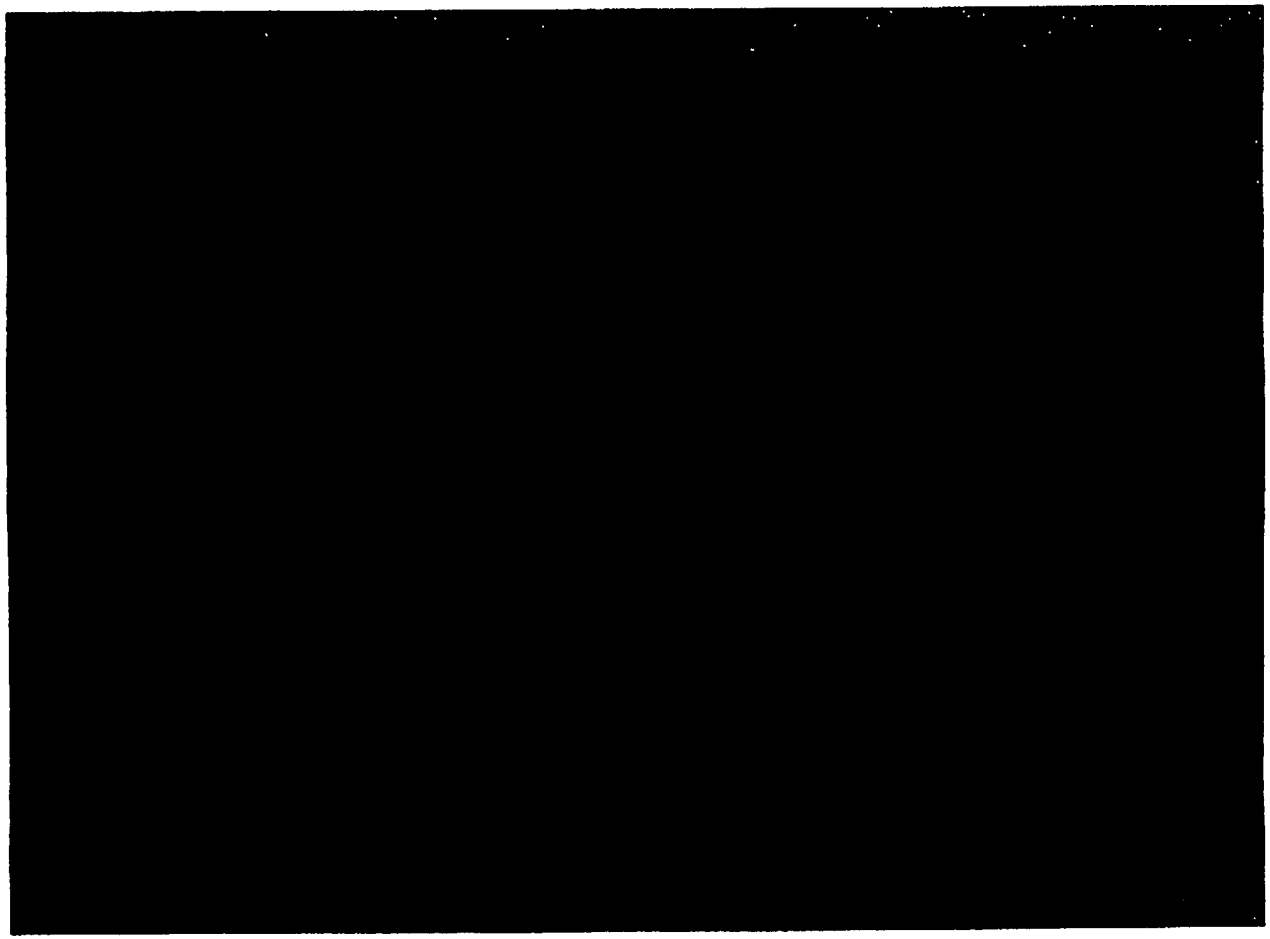


Fig 13 **F.** HUVEC on matrigel (X40)

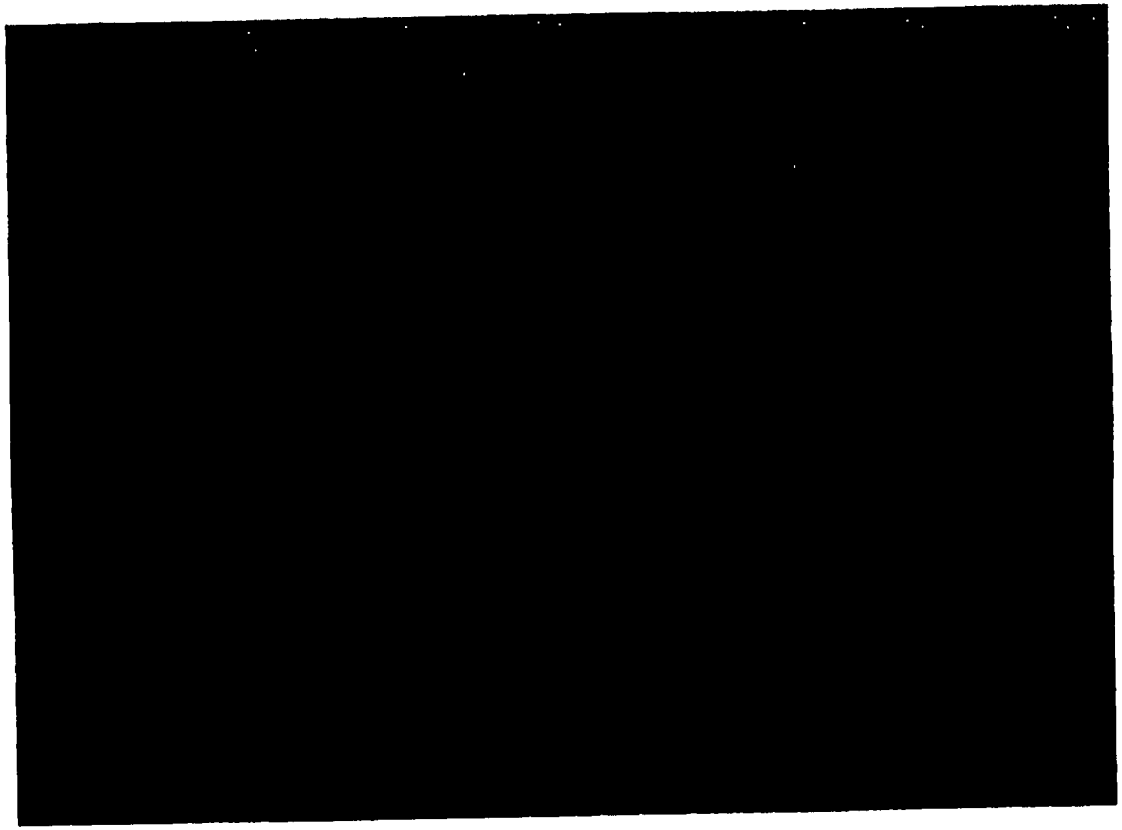


Fig 13 G. :HUVEC on matrigel+FGF
(x40)

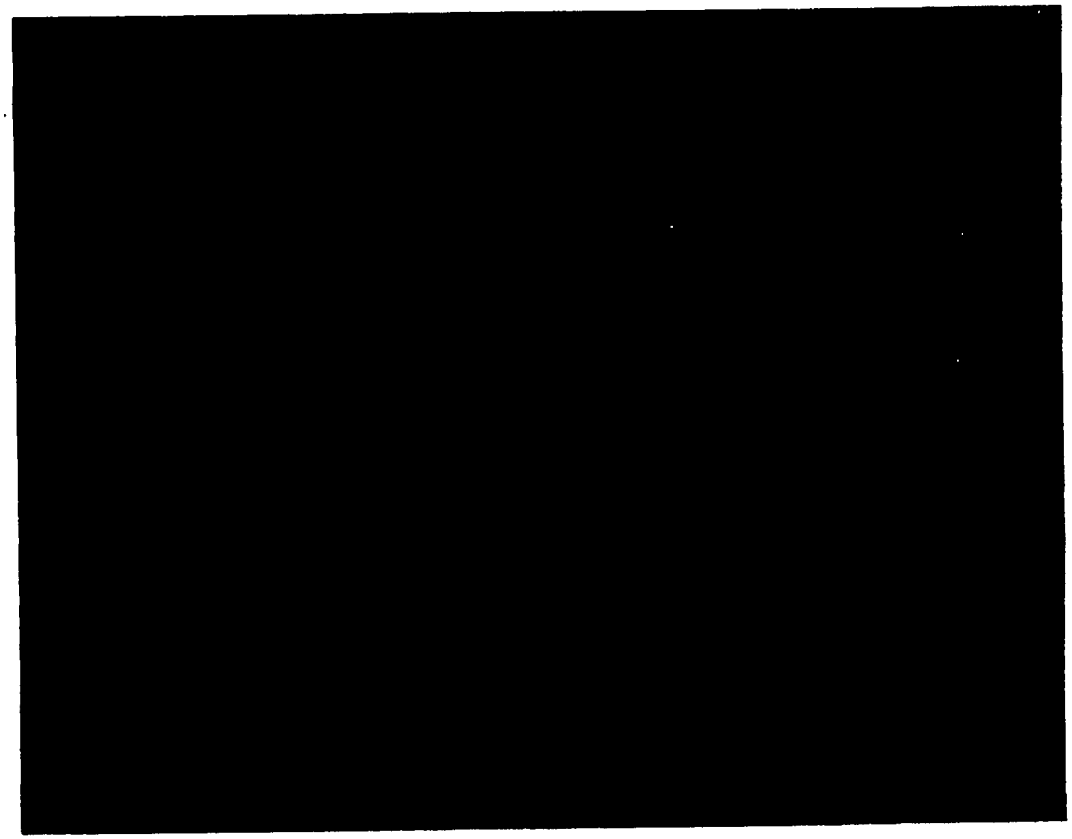


Fig 13 **H. HUVEC on matrigel + YR (x40)**

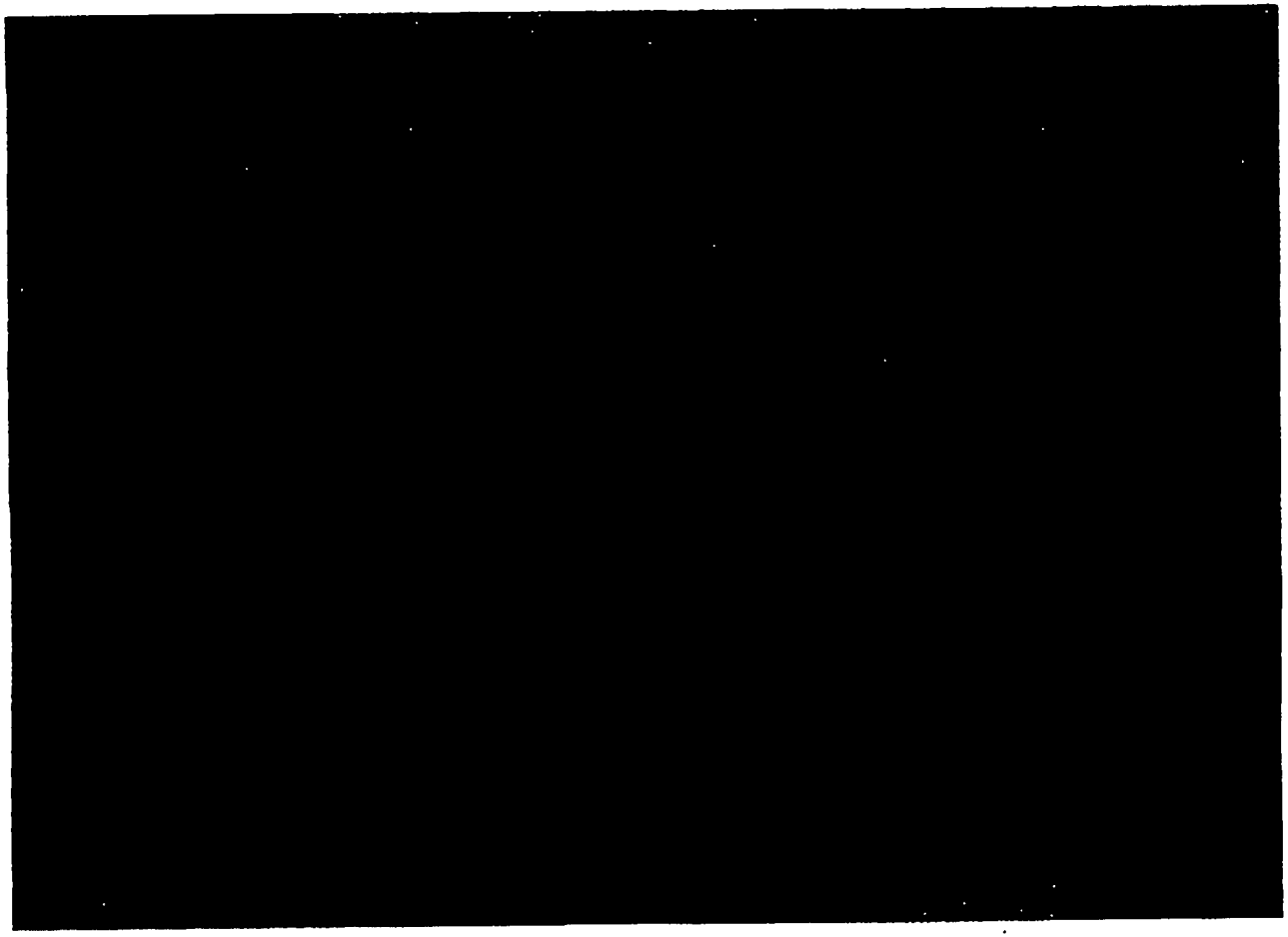


Fig 13 I. **HUVEC on matrigel + QF**
(X40)

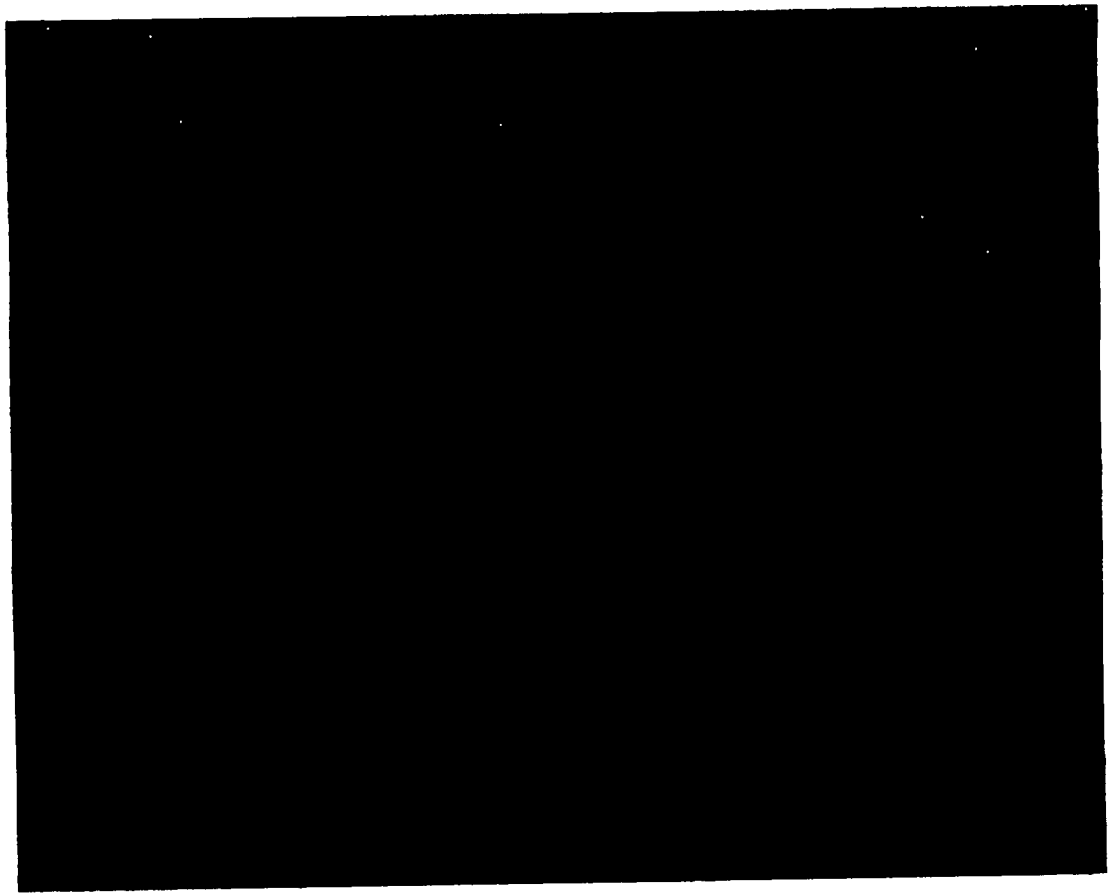
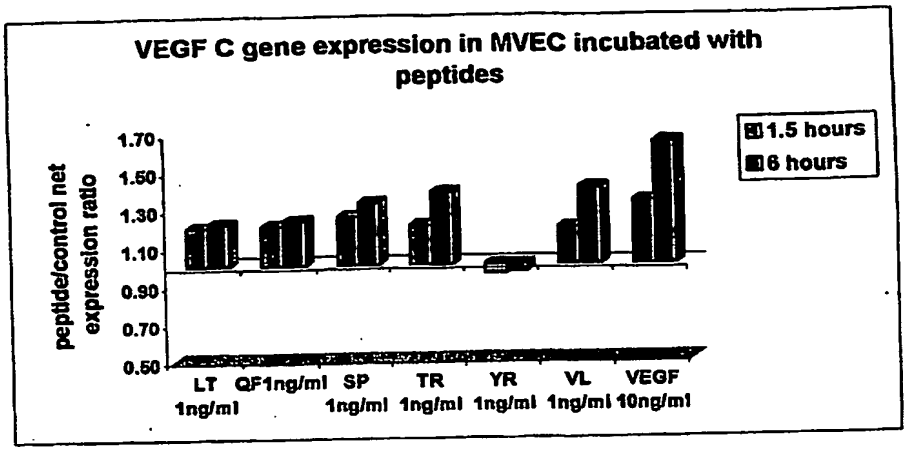
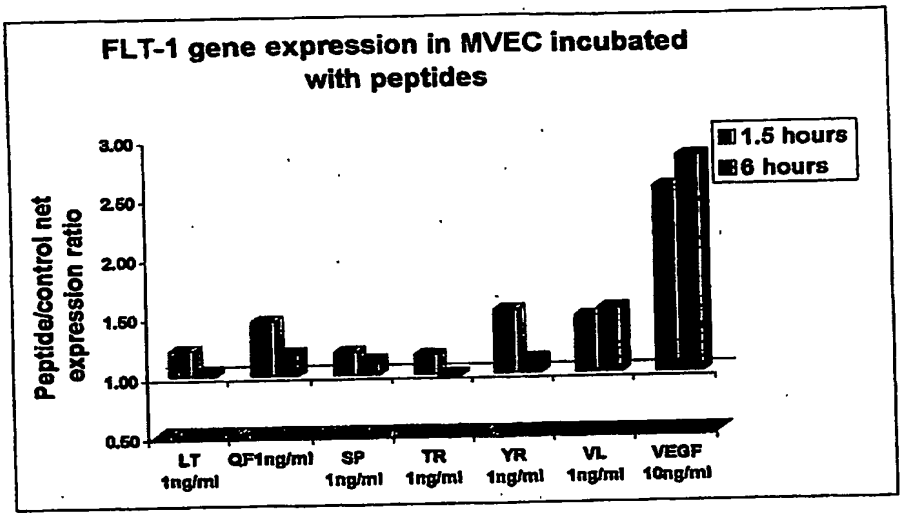


Fig 13 **J. HUVEC on matrigel + VL (x40)**

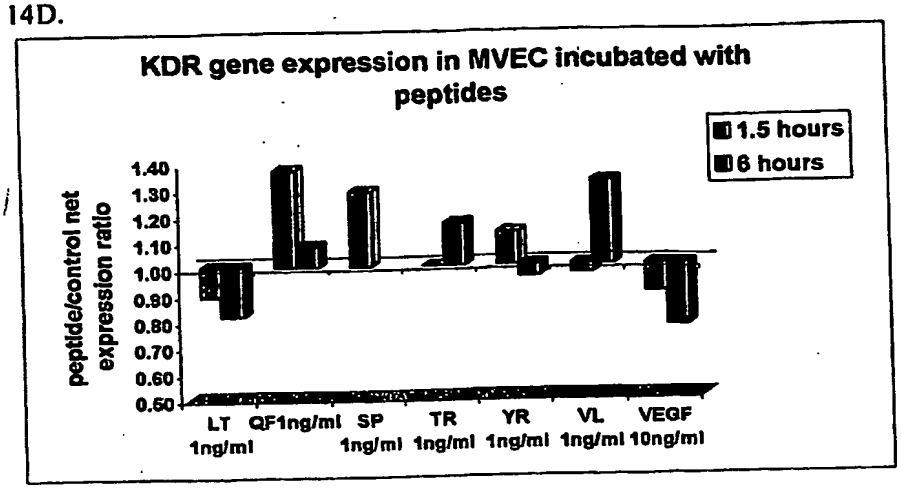
14B.


VEGF C gene expression in MVEC incubated with peptides
peptide/control net expression ratio
1.70
1.50
1.30
1.10
0.90
0.70
0.50
1.5 hours
6 hours
LT 1ng/ml
QF1ng/ml
SP 1ng/ml
TR 1ng/ml
YR 1ng/ml
VL 1ng/ml
VEGF 10ng/ml

14C.


FLT-1 gene expression in MVEC incubated with peptides
Peptide/control net expression ratio
3.00
2.50
2.00
1.50
1.00
0.50
1.5 hours
6 hours
LT 1ng/ml
QF1ng/ml
SP 1ng/ml
TR 1ng/ml
YR 1ng/ml
VL 1ng/ml
VEGF 10ng/ml

14D.


KDR gene expression in MVEC incubated with peptides
1.5 hours
6 hours
peptide/control net expression ratio
1.40
1.30
1.20
1.10
1.00
0.90
0.80
0.70
0.60
0.50
LT 1ng/ml
QF1ng/ml
SP 1ng/ml
TR 1ng/ml
YR 1ng/ml
VL 1ng/ml
VEGF 10ng/ml

14E.

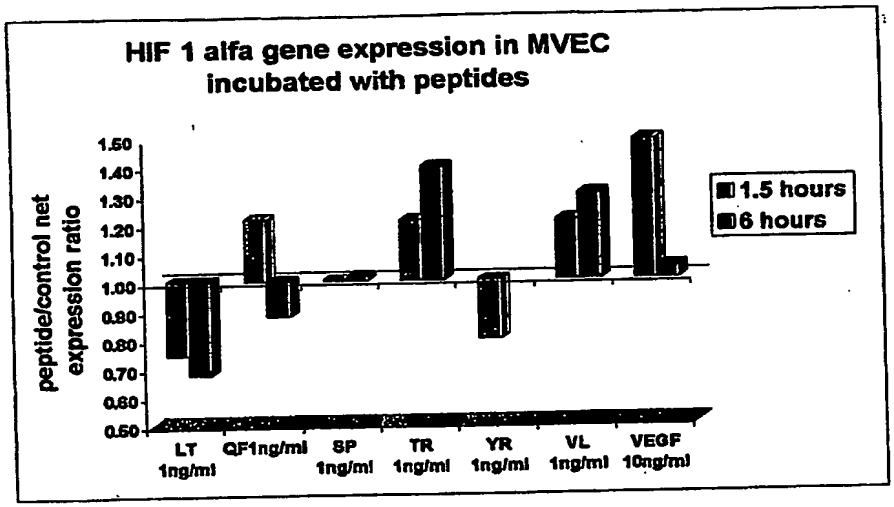
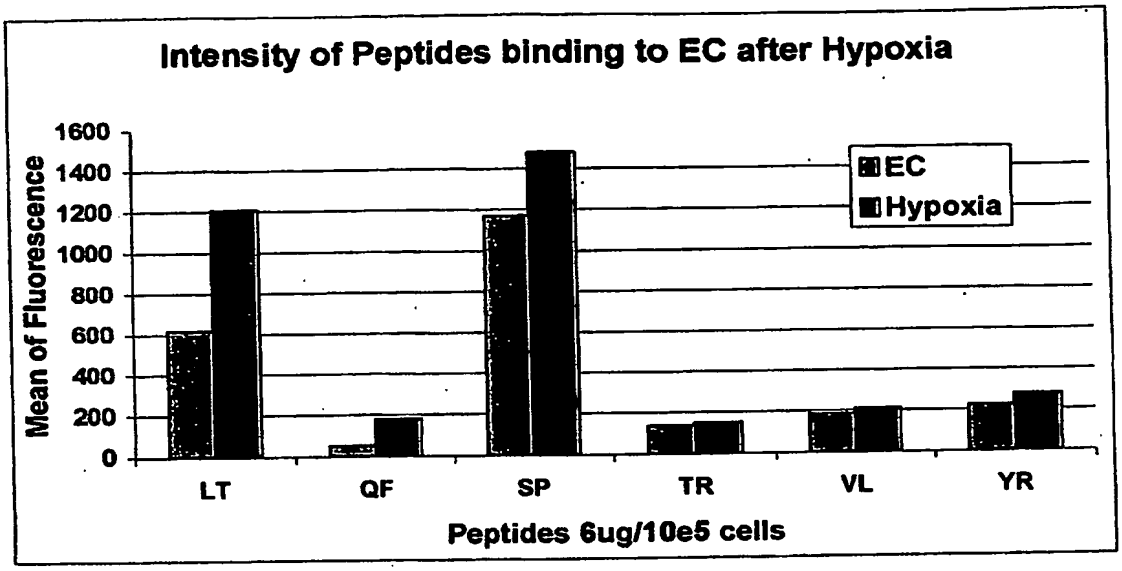
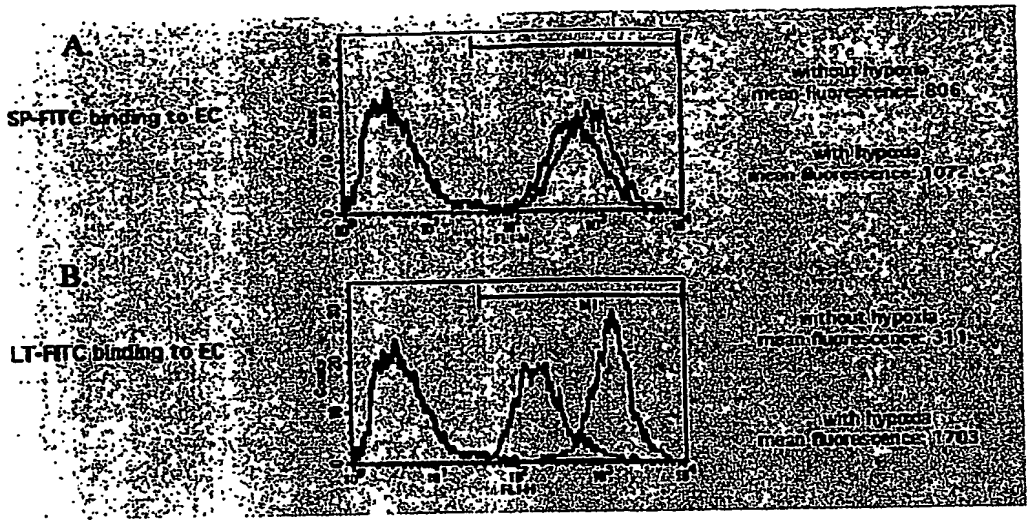

HIF 1 alfa gene expression in MVEC incubated with peptides
1.5 hours
6 hours
peptide/control net expression ratio
1.50
1.40
1.30
1.20
1.10
1.00
0.90
0.80
0.70
0.60
0.50
LT 1ng/ml
QF1ng/ml
SP 1ng/ml
TR 1ng/ml
YR 1ng/ml
VL 1ng/ml
VEGF 10ng/ml

Figure 15.

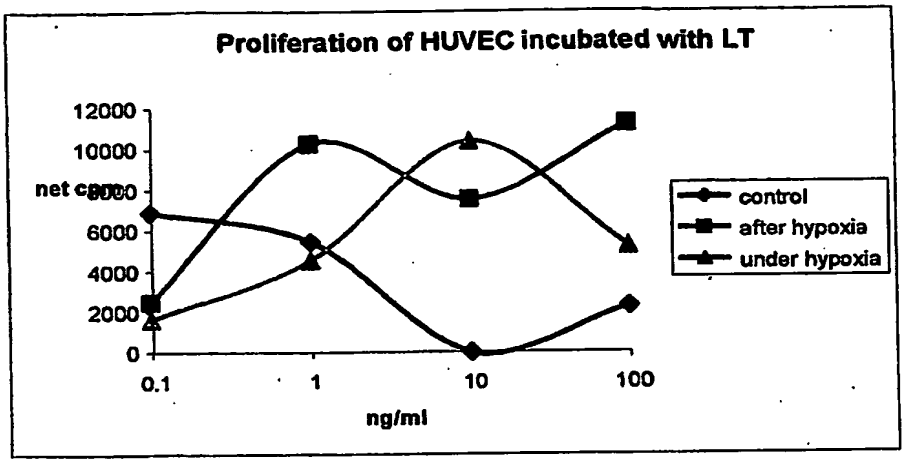

Intensity of Peptides binding to EC after Hypoxia
Mean of Fluorescence
1600
1400
1200
1000
800
600
400
200
0
EC
Hypoxia
LT
QF
SP
TR
VL
YR
Peptides 6ug/10e5 cells

Figures 16a-b

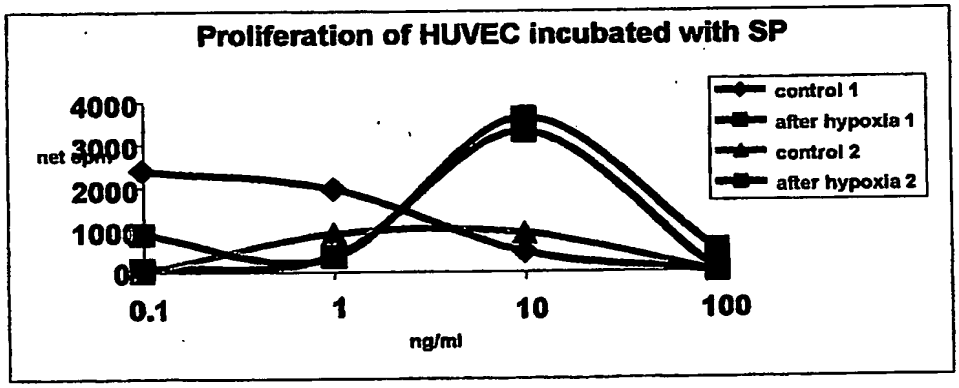


Figures 17a-f

17A.



17B.



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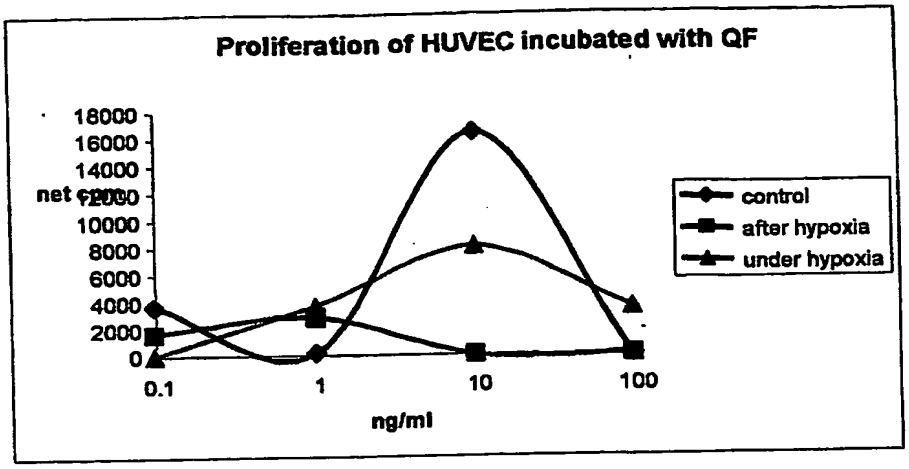
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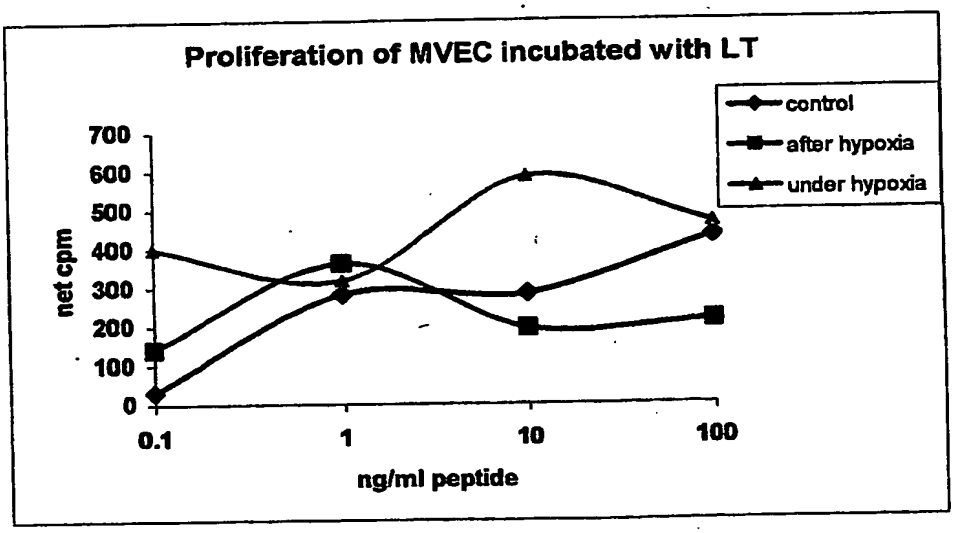
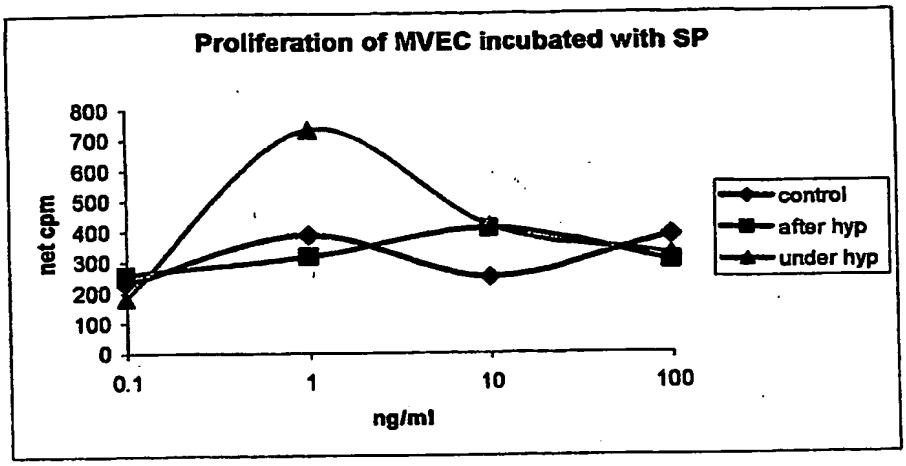
18000
16000
14000
net cpm
10000
8000
6000
4000
2000
0
0.1
1
10
100
ng/ml
control
after hypoxia
under hypoxia

17D.


Proliferation of MVEC incubated with LT
700
600
500
400
300
200
100
0
net cpm
0.1
1
10
100
ng/ml peptide
control
after hypoxia
under hypoxia

17E.


Proliferation of MVEC incubated with SP
net cpm
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700
600
500
400
300
200
100
0
0.1
1
10
100
ng/ml
control
after hyp
under hyp

17F.

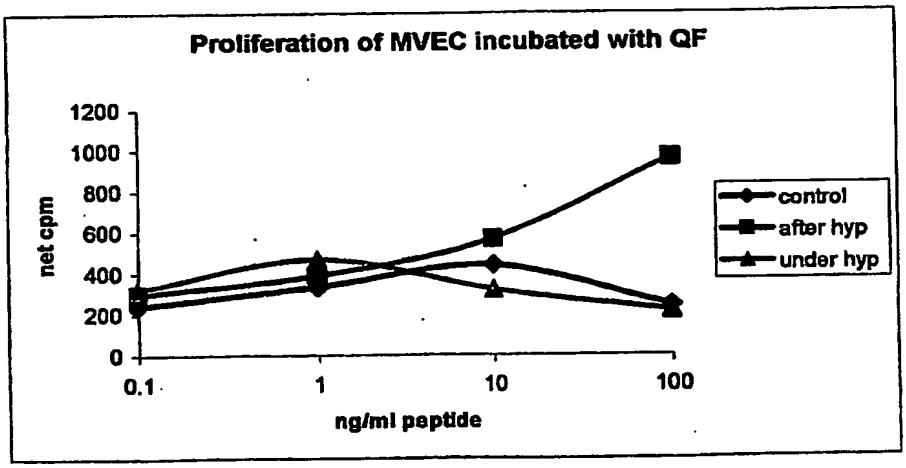

Proliferation of MVEC incubated with QF
net cpm
1200
1000
800
600
400
200
0
0.1
1
10
100
ng/ml peptide
control
after hyp
under hyp

Figure 18a-e.

18A.

HUVEC under hypoxia x100

18B.

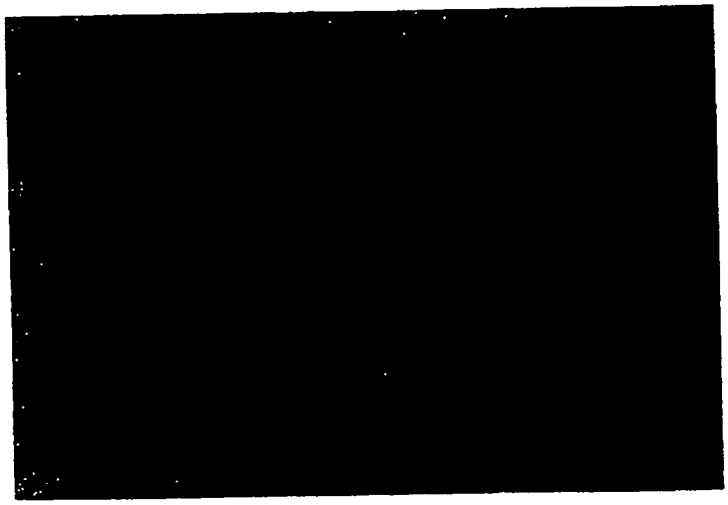
HUVEC + FGF under hypoxia x100

18C.

HUVEC + SP under hypoxia x100

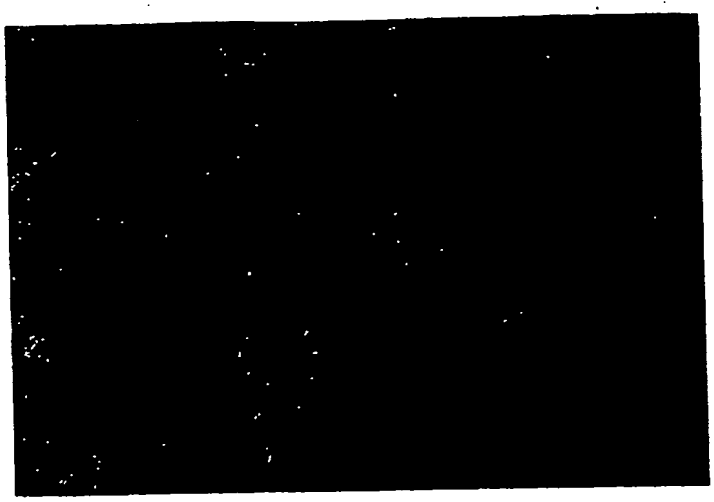
18D.

MVEC + FGF under hypoxia x100



18E.

MVEC + SP under hypoxia x100



Figures 19a-e.

Vascularization

19A.

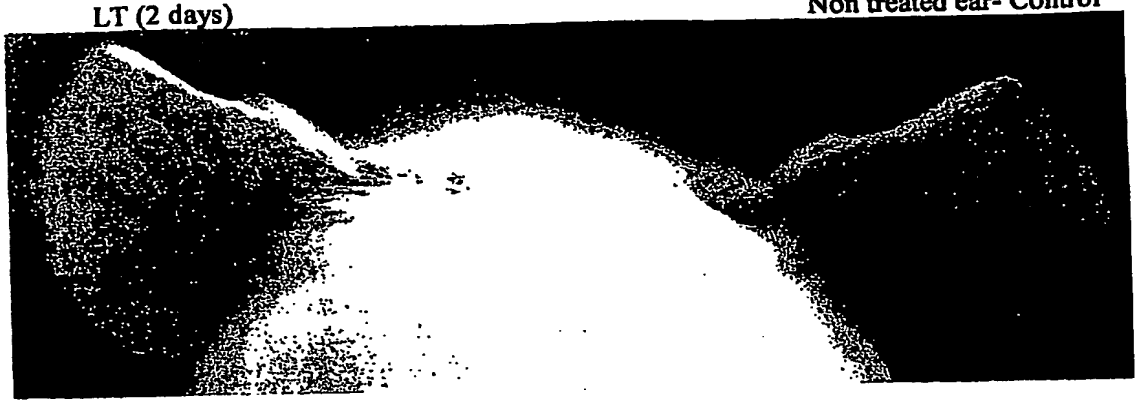
VEGF (positive control)

Non treated ear- Control

19B.

LT (2 days)

Non treated ear- Control



19C.

YR (2 days)

Non treated ear- Control

19D.

QF (2 days)

Non treated ear- Control

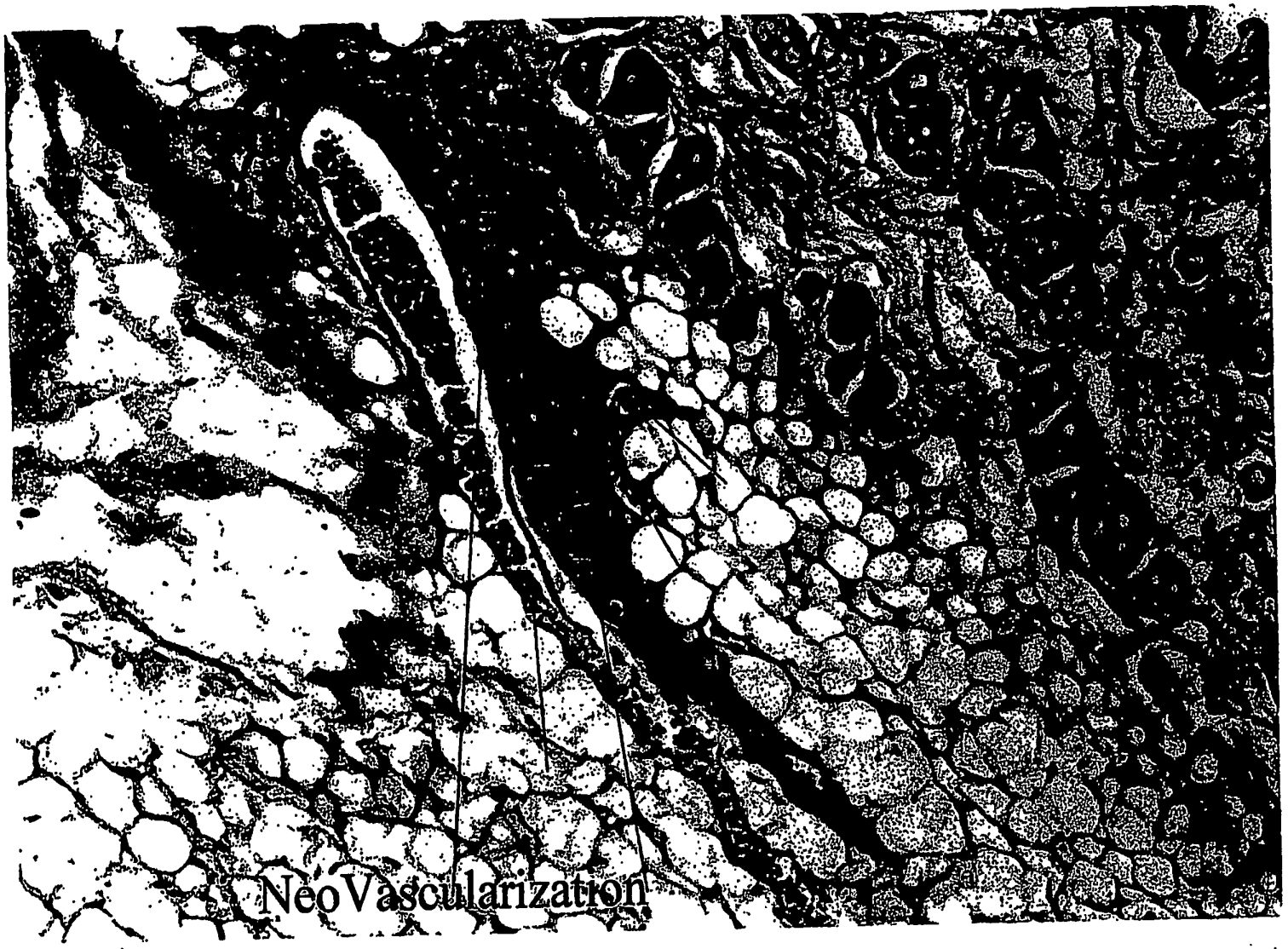
19E.

SP (2 days, 0.1µg/ear XXX)

Non treated ear- Control

Fig. 20a

Fig. 20b



.Figure 21.

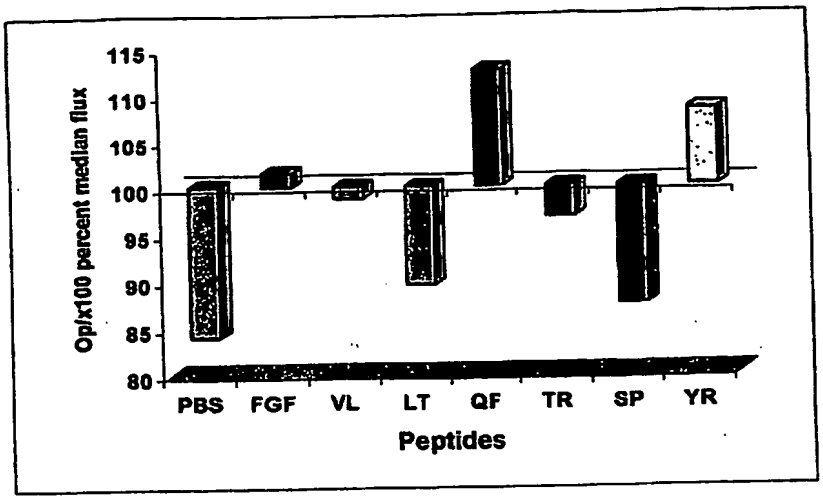

115
110
105
100
95
90
85
80
Op/x100 percent median flux
PBS
FGF
VL
LT
QF
TR
SP
YR
Peptides

Fig. 22

Seq 1: VPWMEPAYQRFL
Seq 2: LLADTTHHRPWT
Seq 3: QPWLEQAYYSTF
Seq 4: SAHGTSTGVPWP
Seq 5: YPHIDSLGHWRR
Seq 6: TLPWLEESYWRP

Seq 1: VPWMEPAYQRFL
Seq 3: QPWLEQAYYSTF
Seq 5: YPHIDSLGHWRR
Seq 6: LPWLEESYWRP

Motif scaned by e-motif
http://dna.stanford.edu/emotif/emotif-scan.html
pw[il][de].y
VEGB MOUSE Vascular endothelial growth factor B precursor
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PVSQ D P HQ+KVV WIDVY RATCQPREVVVPL
PVSQPDAPGHQRKVVSWIDVYTRATCQPREVVVPL
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Seq 3: QPWLEQAYYSTF
Seq 5: YPHIDSLGHWRR
Seq 6: LPWLEESYWRP

SEQUENCE LISTING

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Battler, Alexander
Raiter, Annat
Weiss, Chana

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